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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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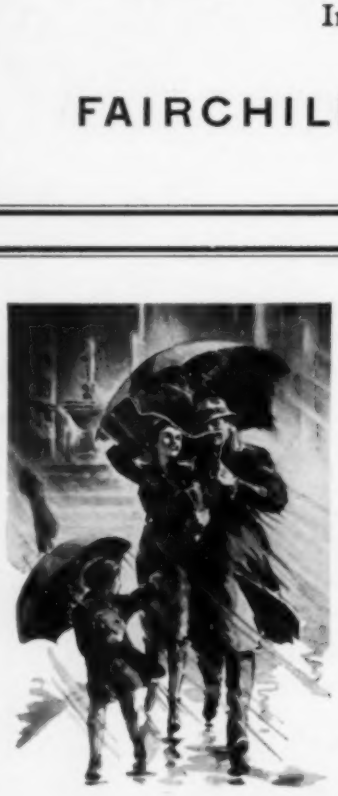

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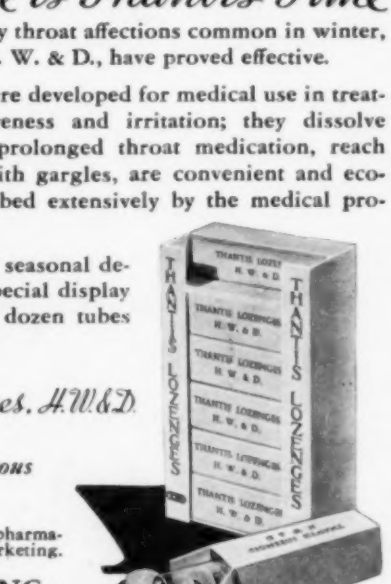

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

A. G. DuMez, Editor, Baltimore, Maryland

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THE SCIENTIFIC AND PRACTICAL PHARMACY EDITIONS OF THE JOURNAL

Beginning with the present month, the JOURNAL OF THE ASSOCIATION will be issued in two parts; a Scientific Edition which will be limited to scientific articles, reports and abstracts, and a Practical Pharmacy Edition in which all other matters of interest will appear. Both editions will be of the same page size to facilitate binding, storage and the interchange of material. The Scientific Edition will be edited by A. G. DuMez and will be distributed about the fifth of each month; the Practical Pharmacy Edition will be edited by E. F. Kelly and distributed about the twentieth of each month.

Both Editions will be sent to the present members of the ASSOCIATION until December 31, 1940, after which time experience may necessitate a change in this plan. In addition, the Practical Pharmacy Edition will be sent to all other dues-paid members of the state pharmaceutical associations during 1940.

This program has been developed carefully and has several objectives. It is expected that the Scientific Edition will be of greater value to those interested in the scientific phases of pharmacy since it will be devoted solely to the publication of scientific articles, reports and abstracts to which increased space and special attention can be given. It is also expected that the Practical Pharmacy Edition will appeal strongly to the practicing pharmacists in every branch of the profession since it will deal with their professional problems, will serve them in promoting professional service of every type and will give publicity to the activities of the ASSOCIATION, as well as to other professional trends and events of national importance in the field which the A. PH. A. serves. Under this arrangement, those connected with pharmacy may make use of either or both editions as their interests may decide, which should result in a more satisfactory and helpful publication service.

For several years, there has been an increased request that the ASSOCIATION arrange to send some publication to the members of the state pharmaceutical associations in order to give them a direct contact with the A. PH. A. and to keep them informed about the activities and objectives of the ASSOCIATION. It was, therefore, decided to send the Practical Pharmacy Edition to these members to determine if it will serve this purpose satisfactorily. It is hoped that the publication will be of real help to the state pharmaceutical associations and that it will bring about a closer knit and stronger professional organization which is needed now and will be needed even more in the future.

E. F. KELLY, *Secretary*
A. G. DUMEZ, *Chairman*
Publication Committee

Tutin: Its Pharmacological Action and Its Antagonism with Sodium Amytal*

By Edward E. Swanson

Several species of the genus *Coriaria* are known to be poisonous to livestock and men. In a previous communication (1), we reported the isolation and pharmacological action of coriamyrtin, a toxic principle from the leaves and stems of *Coriaria myrtifolia*. This species grows in Southern France and other countries bordering the Mediterranean Sea. Maloney (2), using our material, made a similar investigation of coriamyrtin. In 1900, Easterfield and Aston (3) isolated a toxic glucoside named tutin from three *Coriaria* species, *C. thymofolia*, *C. ruscifolia* and *C. augustissima*. These plants are indigenous to New Zealand. The pharmacological action of tutin has been reported by Easterfield and Aston (3), (4), Fitchett and Malcohm (5), Christie (6) and Marshall (7). Their results indicate that it is a convulsant drug similar to picrotoxin and coriamyrtin.

Our interest in coriamyrtin led us to extend our studies with tutin. Through the generous coöperation of Dr. B. C. Aston, Chief Chemist of the New Zealand Department of Agriculture, Wellington, New Zealand, we were in possession of a shipment of *Coriaria thymofolia* which he personally collected and dried. In addition, Dr. Aston was kind enough to send us a sample of his impure tutin which could be easily purified and recrystallized.

EXPERIMENTAL

Isolation of Tutin.—The isolation of tutin was achieved by percolating the pulverized plant with ethyl alcohol. Upon evaporation, the syrup was diluted with water. Tutin was then extracted with ether from the aqueous solution, and it was finally crystallized from ethyl alcohol. The pure substance, in oblique-ended prisms, is more soluble in ether than chloroform, soluble in hot alcohol, less soluble in cold alcohol and slightly soluble in water. It melts at 215–216° C. (corrected) and has a specific rotation $[\alpha]_D^{25} = +50°$ in chloroform. Analytical data and molecular weight determinations suggest the empirical formula, $C_{20}H_{24}O_8$ (calculated C 61.35, H 5.93, mol. wt. 391; found C 61.36, 61.32, H 6.03, 5.96, mol. wt. 418, 421). Easterfield and Aston (3) reported the empirical formula, $C_{17}H_{20}O_7$ (calculated C 60.7, H 5.9, mol. wt. 336; found C 60.8, H 6.0, mol. wt. 334, 327), with a melting point of 208–209° C. Tutin when boiled with dilute hydrochloric acid reduces Fehling's solution, thus confirming that it is a glucoside (glycoside). For pharmacological experiments, a stock solution of 1:1000 tutin in approximately 10 per cent alcohol was employed, and necessary dilutions were made from it when needed.

Convulsions, Toxicity and Other Effects of Tutin.—When injected into the abdominal lymph sac of frogs, tutin in the dosage of 0.002 mg. per Gm. or more caused convulsions in approximately 30–50 minutes. Coriamyrtin (1) produced convulsions in 13 minutes with 0.001 mg. per Gm. by the same route of administration. Thus, in frogs tutin is considerably slower acting than coriamyrtin. The convulsions of tutin were clonic in type, with head drawn back and legs extended. Tutin convulsions were less severe and less frequent than those induced by coriamyrtin.

As with coriamyrtin (1), the origin of convulsions due to tutin is also apparently in the medulla and upper cord, because decapitation in frogs did not stop the convulsion, whereas destruction of the medulla and upper cord abolished their further occurrence.

In rats and rabbits, tutin in sufficient amounts, given either intravenously or subcutaneously, produced clonic convulsions, with the head retracted and legs extended. The animals, particularly rabbits, assumed an opisthotonic posture. External stimuli did not appear to have much influence upon the onset of convulsions.

Comparisons were made with other convulsants—coriamyrtin, picrotoxin, metrazol and thujone.

Table I summarizes the minimal convulsive doses (M. C. D.), the time of onset of convulsions and the minimal lethal doses (M. L. D.) in mice and rabbits following intravenous and subcutaneous injections. From the same data, the median convulsive dose ($CD_{50} \pm$ Standard Error) and the median lethal dose ($LD_{50} \pm$S. E.) could also be computed according to the formula of Bliss (8). The ratio between M. L. D. and M. C. D. varies according to the drug as well as the mode of administration.

As also shown in Table I, coriamyrtin has a most prompt action following an M. C. D., while tutin requires the longest latent period preceding the onset of convulsions. The remaining drugs, picrotoxin, metrazol and thujone, have an intermediate speed in the development of convulsions. When the animals survived, recovery after tutin took place more slowly than after other convulsants studied.

Tutin was found to increase the amplitude and rate of respiration, raise the blood pressure, slightly accelerate the heart rate, have very slight effect on isolated rabbits' uteri and intestines, and cause no

* From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

changes in the caliber of frogs' leg vessels by perfusion with a 1:100,000 solution. These results are comparable to those with coriamyrtin as reported previously (1).

took place. Rabbits were used throughout the experiments.

From Table II, it should be noted that "Sodium Amytal" saved 4 out of 5 animals receiving 55

TABLE I.—TOXICITY OF TUTIN IN COMPARISON WITH CORIAMYRTIN, PICROTOXIN, METRAZOL AND THUJONE

Compound	Species of Animals	Number of Animals Used	Method of Administration	Observed M. C. D. Mg. per Kg.	Observed M. L. D. Mg. per Kg.	Onset of Convulsions after M. C. D., Minutes	Computed CD_{50} ± S. E. Mg. per Kg.	Computed LD_{50} ± S. E. Mg. per Kg.
Tutin	Mice	153	Subcutaneous	1.60	4.00	35	1.226 ±0.202	3.613 ±0.309
	Rabbits	39	Subcutaneous	0.80	1.50	79	0.709 ±0.040	1.521 ±0.073
		30	Intravenous	0.50	1.25	46	0.448 ±0.034	1.244 ±0.100
Coriamyrtin	Mice	89	Subcutaneous	0.90	3.50	9	0.816 ±0.048	3.234 ±0.261
	Rabbits	30	Subcutaneous	0.30	1.20	10	0.291 ±0.024	0.930 ±0.045
		48	Intravenous	0.14	0.40	1−	0.129 ±0.006	0.371 ±0.026
Picrotoxin	Mice	90	Subcutaneous	3.00	11.00	20	2.815 ±0.267	10.52 ±0.920
	Rabbits	45	Subcutaneous	2.00	2.50	48	1.098 ±0.154	1.956 ±0.176
		48	Intravenous	0.50	1.25	20	0.450 ±0.048	1.016 ±0.059
Metrazol	Mice	175	Subcutaneous	60.0	114.0	18	58.80 ±2.310	110.10 ±3.050
	Rabbits	33	Subcutaneous	50.0	87.5	15	44.82 ±5.44	76.00 ±3.390
		38	Intravenous	11.0	80.0	1+	10.43 ±0.41	74.73 ±2.460
Thujone	Mice	45	Subcutaneous	1.25 cc.	3.00 cc.	18	1.094 ±0.109	2.157 ±0.331
	Rabbits	50	Subcutaneous	0.30 cc.	0.40 cc.	16	0.181 ±0.043	0.362 ±0.070
		52	Intravenous	0.003 cc.	0.035 cc.	1	0.00235 ±0.00031	0.031 ±0.001

Detoxification of Tutin in Comparison with Coriamyrtin, Picrotoxin, Metrazol and Thujone by "Sodium Amytal."—In view of the fact that picrotoxin and coriamyrtin (1) are detoxified by certain barbiturates, it might be expected that "Sodium Amytal" (Sodium Iso-amyl Ethyl Barbiturate, Lilly) could combat the excessive doses of tutin, metrazol and thujone. The same technique, first elaborated by Barlow (9) for strychnine poisoning, was adopted in the present investigation; that is, the convulsant substances were injected subcutaneously, and "Sodium Amytal" was administered by vein and repeated when recurrence of convulsions

M. L. D.'s of tutin (75 mg. per Kg.), but none of the rabbits was saved with 60 M. L. D.'s. As previously shown (1), 45 M. L. D.'s of coriamyrtin and 40 M. L. D.'s of picrotoxin were detoxified by "Sodium Amytal." Thus, more lethal doses of tutin were antidoted by "Sodium Amytal" than with coriamyrtin or picrotoxin. It may be pointed out that in tutin poisoning more "Sodium Amytal" was needed in order to obtain successful results, which is undoubtedly due to its protracted course of intoxication. The practical application of this antagonism to the treatment of poisoning by *Coriaria thymofolia* with "Sodium Amytal" is only obvious.

TABLE II.—DETOXIFICATION OF TUTIN BY "SODIUM AMYTAL" IN COMPARISON WITH OTHER CONVULSANTS IN RABBITS

Drug	Quantity Given Subcutaneously, Mg. per Kg.	Equivalent to Number of M. L. D.'s	Average Amount of "Sodium Amytal" Injected Intravenously, Mg. per Kg.	Number of Animals Used	Number of Animals Died
Tutin	52.5	35	508.30	3	0
	60.0	40	575.00	3	0
	67.5	45	616.60	3	0
	75.0	50	625.00	3	0
	82.5	55	650.00	5	1
	90.0	60	533.30	3	3
Coriamyrtin	48.0	40	129.2	3	0
	54.0	45	132.5	5	2
	60.0	50	145.8	3	3
Picrotoxin	87.5	35	153.0	5	0
	100.0	40	210.0	5	1
	112.5	45	234.5	5	4
Metrazol	218.75	2½	75.0	5	0
	437.50	5	150.0	5	0
	656.25	7½	225.0	5	2
	875.00	10	250.0	3	3
Thujone	1.0 cc.	2½	40.19	5	0
	2.0 cc.	5	45.00	5	1
	3.0 cc.	7½	57.75	5	4
	4.0 cc.	10	60.20	5	5

In the detoxification of metrazol and thujone, the results were not so successful as with tutin, coriamyrtin or picrotoxin. As shown in Table II, only $7^1/_2$ M. L. D.'s of metrazol and 5 M. L. D.'s of thujone could be tolerated with the help of "Sodium Amytal." According to Fischer and Löwenback (10), metrazol has a pronounced effect on the cerebrum. Sparks (11) found that thujone also acts on the cortex. Picrotoxin, coriamyrtin and tutin, all have an action on the medulla. It appears that "Sodium Amytal" is more effective in combating convulsants acting upon the medulla than those acting upon the brain.

Detoxification of "Sodium Amytal" by Tutin.—In view of the fact that there is a mutual antagonism between certain convulsants and barbiturates, it might be expected that tutin could detoxify lethal doses of barbiturates, such as "Sodium Amytal." In barbiturate poisoning, the value of picrotoxin has been repeatedly demonstrated by Maloney (12), (13), (14) and Koppanyi and his associates (15). In a previous communication, we showed that in rabbits coriamyrtin or picrotoxin can detoxify $1^3/_4$ but not 2 M. L. D.'s of "Sodium Amytal." With coriamyrtin Maloney (2) reported a similar action against three different barbiturates. In the present study, "Sodium Amytal" was injected subcutaneously rather than intraperitoneally, as previously administered (1), because of the slow onset of action of tutin. Repeated doses of tutin were given by vein to keep the respiration stimulated and reflexes active.

TABLE III. DETOXIFICATION OF "SODIUM AMYTAL" BY TUTIN IN RABBITS

Quantity of "Sodium Amytal" Subcutaneously, Mg. per Kg.	Equivalent to Number of M. L. D.'s of "Sodium Amytal"	Average Quantity of Antidote by Vein, Mg. per Kg.	Number of Animals Used	Number of Animals Died
150	1	6.25	1	0
200	$1^1/_2$	9.375	3	0
250	$1^2/_3$	15.625	3	0
300	2	19.52	3	0
350	$2^1/_2$	23.43	3	1
400	$2^2/_3$	20.00	3	3

As shown in Table III, tutin saved all animals receiving 1 and 2 M. L. D.'s of "Sodium Amytal." Two out of 3 also recovered from $2^1/_2$ M. L. D.'s of "Sodium Amytal" by tutin treatment, but all 3 animals died from $2^2/_3$ M. L. D.'s. It is very obvious from the above results that "Sodium Amytal" is far more effective in antidoting tutin than vice versa.

SUMMARY

1. Tutin, $C_{20}H_{23}O_8$, a glycoside of *Coriaria thymofolia*, has a convulsant action similar to that of coriamyrtin and picrotoxin. It is slower in the onset of action but longer in duration.

2. Comparisons have been made with coriamyrtin, picrotoxin, metrazol and thujone, in mice and rabbits, by the determination of convulsive and lethal doses, injected subcutaneously and intravenously.

3. In rabbits "Sodium Amytal" can detoxify 55 minimal lethal doses of tutin, 45 minimal lethal doses of coriamyrtin, 40 minimal lethal doses of picrotoxin, but only 7.5 minimal lethal doses of metrazol and 5 minimal lethal doses of thujone.

4. Conversely, tutin given by vein can detoxify in rabbits a little more than 2 minimal lethal doses of "Sodium Amytal" injected subcutaneously.

The author is indebted to Dr. K. K. Chen for his valuable suggestions during the investigation and his assistance in preparation of the manuscript. Thanks are also due to Messrs. W. E. Fry, E. Brown Robbins and Herbert Glick for their technical assistance in this work.

REFERENCES

(1) Swanson, *J. Pharmacol. & Exper. Therap.*, 57, 410 (1936).

(2) Maloney, *Ibid.*, 57, 361 (1936).

(3) Easterfield and Aston, *New Zealand Dept. of Agri.*, Part I, 8 (1900).

(4) Easterfield and Aston, *Ibid.*, Part II, 4 (1901).

(5) Fitchett and Malcohm, *Quart. J. Exper. Physiol.*, 2, 335 (1909).

(6) Christie, *New Zealand Med. J.*, 3, 211 (1889–1890); 4, 1 (1890–1891).

(7) Marshall, *J. Pharmacol. & Exper. Therap.*, 4, 135 (1912–1913).

(8) Bliss, *Quart. J. Pharm. & Pharmacol.*, 11, 192 (1938).

(9) Barlow, *J. A. M. A.*, 98, 1980 (1932).

(10) Fischer and Löwenback, *Arch. exptl. Path. Pharmakol.*, 174, 502 (1934).

(11) Sparks, *Arch. internat. de pharmacodyn. et de thérapie*, 33, 460 (1927).

(12) Maloney, Fitch and Tatum, *J. Pharmacol. & Exper. Therap.*, 41, 465 (1931).

(13) Maloney and Tatum, *Ibid.*, 44, 337 (1932).

(14) Maloney, *Ibid.*, 49, 133 (1933); *J. Nat. M. A.*, 25, 47, 90 (1933).

(15) Linegar, Dille and Koppanyi, *Science*, 82, 376 (1935); *Proc. Soc. Exptl. Biol. & Med.*, 33, 396 (1935–1936).

Toxicity of Triethylene Glycol and the Effect of Para-Amino-Benzene-Sulfonamide upon the Toxicity of This Glycol

*By W. M. Lauter and V. L. Vrla**

The recent investigations of Kesten, Mulinos and Pomerantz (1) on the toxicity of diethylene glycol, of Holck (2) on glycerine, ethylene glycol and the work done by Geiling and others (3) while investigating the toxicity of solutions of *p*-amino-benzene-sulfonamide in diethylene glycol solutions made it seem that "the ether linkage of the di-glycols may be the portion of the molecule responsible for the degeneration of epithelial cells of parenchymatous organs, especially of the kidney." (Kesten and others.) It was therefore interesting to determine the toxicity of triethylene glycol ($HO.C_2H_4OC_2H_4OC_2H_4OH$) which has two ether linkages in the molecule. It might be expected that its toxicity would be greater than that of the diethylene glycol with only one ether linkage.

Haag and Ambrose (5) stated that concentrations of 3 and 10 per cent, respectively, of diethylene glycol in the drinking water of rats proved rapidly fatal. Holck (2) states that when rats drank from a 5 per cent solution, the average duration of life was only eight days. This author also states that even in a concentration of 0.25% some indication of impaired growth was detectable. He also states that pregnancy did not occur when males and females were mixed and both received 0.5% of pure diethylene glycol in the drinking water.

* From the Research Department, R. J. Strasenburgh Co., Rochester, New York.

EXPERIMENTAL

The triethylene glycol used (commercial grade obtained from the Carbide and Carbon Chemicals Corp.) is a colorless liquid, has a specific gravity of 1.125 at 20° C. and boils at 288° C. It is hygroscopic. It is a good solvent for *p*-amino-benzene-sulfonamide. Ten per cent solutions remained clear at low temperatures.

CHART I.—TOXICITY OF TRIETHYLENE GLYCOL ADMINISTERED BY STOMACH TUBE

Expt. No.	No. of Rats	Cc. Triethylene Glycol per Kg. Body Weight, Daily Dosage	Method of Administration	Time of Treatment, Days	Time of Post-Observation, Days	Weight Increases During Treatment	Weight Increases During Post-Treatment Period	Remarks
A	5	0.1	As 5% aqueous solution	30	15	Normal	Normal	Animals looked healthy. All females had normal litters. No deaths
B	5	3.0	As 30% aqueous solution	30	15	Normal	Normal	Animals looked healthy. Females (2) had litters, one of which was small. No deaths
C	5	10.0	Undiluted	30	15	Slow	Slow during first week, but increased in 2nd week	Animals showed definite toxic symptoms, such as loss of hair and diarrhea. One female had a small litter. No deaths
D	5	20.0	Undiluted	30	15	3 animals died within 24 hours, 2 within 48 hours		All animals died. Toxic symptoms were severe. Food was refused, the animal lay on its side, respirations increased in rapidity and depth. The fur became ruffled

Albino rats (100-210 Gm. weight) were used. A standard laboratory feed preparation (Purina Mills) was employed. The triethylene glycol was given by the stomach tube daily for 30 days in succession and the post-treatment observation period lasted 15 days.

Chart I shows that rats will not be killed by a dosage of 10 cc. per Kg. body weight, although the toxic symptoms are pronounced. In contrast to diethylene glycol, rats receiving 3 cc. per Kg. body weight had litters, and even one female of the 5 cc. per Kg. body weight had a litter, although small.

CHART II.—TOXICITY OF TRIETHYLENE GLYCOL IN AQUEOUS SOLUTION

Expt. No.	No. of Rats	Weight of Rats, Gm.	Concentration in Water	Time of Treatment, Days	Time of Post-Observation, Days	Weight Changes	Remarks
E	5	140–198	5% by vol. = 5.6% by weight	30	15	No increase in weight in all animals. Those that died refused food and lost weight. The survivors showed increase beginning the 39th day	1 animal died on the 8th day 1 animal died on the 21st day 1 animal died on the 28th day 2 recovered—all showed severe toxic symptoms
F	5	130–196	10% by vol. = 11.3% by weight	30	15	Weight decrease noticeable on the 4th day	2 animals died on the 6th day 1 animal died on the 8th day 1 animal died on the 9th day 1 animal died on the 12th day
G	5	30– 34	3% by vol. = 3.4% by weight	30	15	The animals showed no signs of toxicity symptoms. They weighed from 115–154 Gm. (average 129 Gm.) on the 31st day	The young rats drank correspondingly far larger amounts of fluids than the adult animals. All animals alive
H	5	28– 32	5% by vol. = 5.6% by weight	30	15	The rats gained weight very slowly during the first two weeks. Thereafter improvement in behavior and weight. Weight on the 31st day 78–111 Gm. (average 92 Gm.)	Severe toxic symptoms during the first two weeks. Improvement thereafter. 1 animal died on the 15th day
I	5	29– 34	Water only. Control	30	15	Weight on the 31st day, 96–124 Gm. (average 115 Gm.)	All animals alive

Albino rats were used. Two series were mature rats (130–200 Gm.) and 3 series were 3-week-old rats (28–34 Gm.). A standard laboratory feed preparation (Purina Mills) was employed. The triethylene glycol was given in aqueous solution. The rats were kept on these solutions for 30 days, and for 15 days on water for post-treatment observation.

The rats were kept on a diet of a standard laboratory feed preparation (Purina Mills).

The experiments were divided into four groups:

A. Triethylene glycol given by stomach tube daily for 30 days. (Chart I.)

B. Triethylene glycol given as aqueous solution in the drinking water for 30 days. (Chart II.)

C. Triethylene glycol given as aqueous solution in the drinking water for 30 days to young rats. (Charts II and III.)

D. Triethylene glycol solutions of *p*-aminobenzene-sulfonamide in high, but not lethal dosages given daily by stomach tube to adult rats for 30 days. (Chart IV.)

Group B.—The animals receiving triethylene glycol in the drinking water (adults drinking approximately 30–40 cc. per day) demonstrated that it is apparently immaterial whether this glycol is given undiluted by stomach tube, or whether it is taken with the daily drinking water.

Group C.—The results obtained with this group of animals induced us to see whether the toxicity for aqueous triethylene glycol solution for adult rats which appears to be around 4% would be lower in young animals.

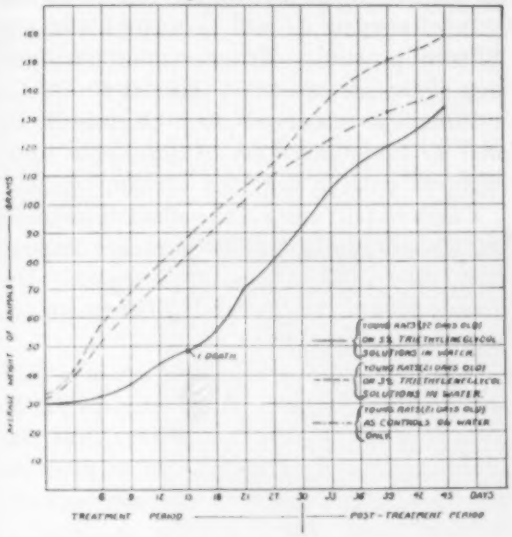
We used three groups of young rats, 21 and 22 days old. It was shown (Charts II and III) that young rats will barely tolerate a 5% concentration, despite the fact that 5 animals drank daily from 120 cc. in the beginning to about 210 cc. after the 22nd day. That would mean from 24–42 cc. daily. The fact that the 3% series progressed splendidly demonstrates again the lower toxicity of triethylene glycol compared with diethylene glycol.

On the basis of previous reports (see above) it seems that triethylene glycol is decidedly less toxic when taken orally than diethylene glycol.

According to Holck, 2.92% solutions of commercial ethylene glycol killed rats usually within 4 days, while propylene glycol in 3.58% solutions was tolerated, but toxic symptoms were observed.

The findings of Geiling and others have shown that diethylene glycol solutions of *p*-amino-benzene-sulfonamide seemed to have no particular effect.

Chart III.—Toxicity of Triethylene Glycol in Aqueous Solution.



We also found this to be the case when this sulfanilamide was dissolved in triethylene glycol.

Chart IV shows that rats tolerated 10% solutions of sulfanilamide in this solvent when they received such large dosages as 1% of their weight daily for 30 days of this solution by stomach tube.

The toxicity of other glycols when given as intramuscular single injections has been described by several authors. We found (see Chart V) that these injections caused considerable local irritation. The lethal dose appears to be approximately 7.5 cc. per Kg. body weight (about 8.4 Gm./Kg. body weight). This places triethylene glycol between propylene glycol and ethylene glycol. Hanzlick (4) shows that the M. L. D. for ethylene glycol is 4.4 Gm./Kg. body weight when given intramuscularly to white rats, while Haag and Ambrose (5) showed that the M. L. D. for diethylene glycol is 7 cc. (7.8 Gm.) per Kg. body weight when injected into the muscles of albino rats. Propylene glycol, however, has a toxicity of 14.7 Gm. per Kg. body weight for intramuscular injections.

CONCLUSIONS

Triethylene glycol is less toxic than diethylene glycol when fed to rats. It is, therefore, doubtful that the ether linkage in the higher glycols is responsible for the increase in toxicity reported previously.

The lethal dosage when given by stomach tube undiluted is between 10 and 15 cc. triethylene glycol per Kg. body weight. Ten cubic centimeters per Kg. body weight show definite toxic symptoms and retarded weight increase.

Water solutions do not seem to be more toxic than when the glycol is given undiluted. Adult rats are killed by 5% solutions while young rats barely survive this concentration. Young rats thrive well on 3% solutions and the increase in weight is absolutely normal. If one considers that as low as 0.25% of diethylene glycol showed impairment of growth in rats, it seems that further investigations of triethylene glycol and glycols of higher molecular weight might be worth while.

The M. L. D. for intramuscular injections for white rats is approximately 8.4 Gm. per Kg. body weight.

CHART IV.—EFFECT OF *p*-AMINO-BENZENE-SULFONAMIDE ON THE TOXICITY OF TRIETHYLENE GLYCOL

Expt. No.	No. of Rats	Weight before Treatment, Gm.	Average Weight before Treatment, Gm.	Average Weight after Treatment, Gm.	Time of Treatment, Days	Time of Post-Treatment Obs., Days	Gm. Sulfanilamide per Kg. Body Weight	Cc. Triethylene Glycol per Kg. Body Weight	Remarks
K	5	138–144	161	189 (30 days) 230 (45 days)	30	15	1	10	No animal died
L	5	124–134	120	122 (30 days) 158 (45 days)	30	15	0.75	7.5	No animal died

A solution of *p*-amino-benzene-sulfonamide in triethylene glycol was given to adult rats by means of a stomach tube One cubic centimeter of this solution contained 0.1 Gm. of the sulfonamide.
Both series of course showed decided sulfanilamide toxicity symptoms, but appeared normal on the 45th day.

CHART V.—TOXICITY OF TRIETHYLENE GLYCOL ADMINISTERED INTRAMUSCULARLY

Expt. No.	No. of Rats	Dosage in Cc. per Kg. Body Weight	Animals Died	Remarks
M	5	5.0 cc. = 5.6 Gm.	None	Toxicity symptoms are slight. Animals appeared normal after 48 hours
N	5	7.5 cc. = 8.4 Gm.	3 died	Animals died within 36 hours. 2 survivors recovered in 3 days
O	5	10.0 cc. = 11.3 Gm.	5 died	Death occurred within 16–36 hours

Triethylene glycol was given intramuscularly into the leg muscles of albino rats (120–145 Gm. weight).

REFERENCES

(1) Kesten, Mulinos and Pomerantz, *J. A. M. A.*, 109, 1509 (1937).
(2) H. G. O. Holck, *Ibid.*, 109, 1517 (1937).
(3) Geiling, Coon and Schoeffel, *Ibid.*, 109, 1532 (1937).
(4) Hanzlick, Seidenfeld and Johnson, *J. Pharmacol. and Exper. Therap.*, 41, 387 (1931).
(5) Haag and Ambrose, *Ibid.*, 59, 93 (1937).

A Preliminary Study of the Anthelmintic Activity *in Vitro* of Fresh Pineapple Juice*

Conrado F. Asenjo†

INTRODUCTION

Recently Berger and the author (1) published a short note pointing to the anthelmintic activity exhibited *in vitro* by fresh pineapple juice.

The purpose of the present paper is to report some further experiments, as well as to summarize the literature related to bromelin, the proteolytic enzyme probably responsible for the digestive activity of the juice.

The existence of a proteolytic enzyme in pineapple juice was discovered by the Venezuelan chemist Marcano (2) in 1891. The name bromelin, derived from *Bromeliaceæ*, the family to which pineapple belongs, was given to this enzyme. Soon afterward a concern in Detroit, Michigan,[1] patented the use of pineapple juice for the preparation of pre-digested foods (3), (4), (5), (6).

Later in that same year (1891) Chittenden (7) learned of Marcano's discovery and undertook a series of experiments with pineapple juice. He showed that the active enzyme is concentrated in the protein precipitate that can be separated by saturation of the juice with ammonium sulfate, sodium chloride or magnesium sulfate. In a second paper (8) published in 1893, particular attention was paid to the nature of the isolated enzyme as well as to its action on different proteins. He reports that inactivation of the enzyme takes place at 60–70° C.

Vines (9) observed that peptolysis as well as fibrin digestion by pineapple juice occurred both in acid and alkaline media.

Caldwell (10) studied the effect of metallic salts upon the action of bromelin, finding that silver salts were most poisonous. He also suggested the presence of two proteolytic enzymes in bromelin.

Willstätter, Grassmann and Ambros (11) showed that bromelin is activated by HCN as well as by H_2S. They report that the optimum cleavage of gelatin takes place at p_H 4.5–5. In the case of albumin peptone the p_H is 5. They describe in detail a method for obtaining very active bromelin preparations.

Ambros and Harteneck (12) claim that the juice from the green fruit is inert toward peptone. According to them the ripe fruit alone yields the activated enzyme. It is their theory that during the process of maturation the natural activator present in the cell juice from the interior of the fruit increases considerably. The inactive enzyme is present in the outer layers of the fruit; the combination of these two principles on crushing the tissue yields then the active bromelin. On the other hand Tanaka (13) found that the largest yields of crude bromelin are obtained from unripe fruits. According to this investigator the proteolytic activity of the juice decreases with increased maturity.

Maschmann (14) has recently studied the effect of different activators on plant proteases including bromelin.

Bergmann and co-workers (15) after experimenting with different synthetic substrates concluded that bromelin contains two enzymes which they have designated as Bromelin I and Bromelin II. These same investigators have effected synthesis using bromelin as promoter (16).

There are records in the literature of the use of pineapple juice as an anthelmintic by the native population of Brazil (17) and India (18). However, up to the present

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[1] Mosquera-Julia Food Co.

time no investigation has been conducted to ascertain the validity of this folk use of pineapple juice.

EXPERIMENTAL

Action of the Juice on Live Parasites.—Common round worms (*Ascaris lumbricoides*) obtained from hogs were incubated at a temperature of 37° C. with fresh pineapple juice, obtained from Cuban pineapples. At the end of 12 hours the parasites were dead and partly digested, while at the end of 24 hours they were totally digested. The results of this initial experiment are well illustrated in the photograph above.

Effect of pineapple juice on *Ascaris* worms: (1) Control in saline solution. (2) Control in pineapple juice heated to 100° C. (3) After 12 hours in fresh pineapple juice. (4) After 24 hours in fresh pineapple juice.

The same experiment was conducted using *Macracanthorynchus hirundinaceus*, another parasite found in hog's intestines, and similar results were obtained.

Minimum Time Necessary for Initial Digestion to Take Place.—Thirty-two lively *Ascaris* were incubated at 37° C. with fresh pineapple juice. Every half-hour four worms were taken out, examined carefully and then dropped into water at 45° C. Living worms when in contact with warm water move actively. At the end of 3 hours the surface of the worms was slightly wrinkled but they were still alive. At the end of three and one-half hours small ulcers were apparent and at the end of four hours large ulcers were visible to the naked eye. The parasites were still alive.

This experiment plainly showed that the digestion of the parasite precedes its death. Apparent signs of digestion can be observed in three to four hours.

Action of Heat on the Proteolytic Activity of the Juice.—The thermal inactivation point of the enzyme present in the juice was found to be in the neighborhood of 65° to 70° C. This is in agreement with the findings of other workers.

The gelatin hydrolysis was measured by means of the formol titration.[1] The decrease in worm

[1] The technique followed in the formol titration throughout this work is the following: 2% gelatin solution containing 1% toluene as a preservative is used as a substrate. To 10 cc. of this gelatin solution 1 cc. of the juice to be tested is added. One-cc. samples are taken from this mixture for titration. The indicator used is a solution containing 0.2% phenolthalein in 50% alcohol. One cc. of 40% formaldehyde is added to each volume of mixture to be titrated. The strength of the sodium hydroxide solution is 0.01*N*. One cc. of this solution is equivalent to 0.14 mg. of amino nitrogen. The tests are run for a period of 24 hours at an incubation temperature of 37° ± 2° C. No buffer is used. The natural p_H of the mixture is in the neighborhood of 4.5 to 5.

TABLE I.—EFFECT OF HEAT ON PROTEOLYTIC ACTIVITY OF JUICE

Temperature in ° C. to Which the Juice Was Pre-heated for 5 Minutes	Action on *Ascaris* after 24 Hours Incubation at 37° C.	Milligrams of Amino Nitrogen Liberated after 24 Hours from 1 Cc. Gelatin Reaction Mixture
25	Digested	0.24
45	Digested	0.28
50	Digested	0.30
55	Digested	0.24
60	Digested	0.22
65	Slight digestion	0.13
70	No digestion	0.01
75	No digestion	0.00

digestive activity runs parallel to the decrease in gelatin hydrolysis.

Dilution at Which the Juice Will Digest Ascaris.—*Ascaris* is digested at a concentration as low as 15 per cent juice. Worms incubated in solutions containing 10 per cent juice were not digested after 24 hours. Distilled water was used as diluent.

Anthelmintic Activity of Juices from Different Geographical Origins.—Juices obtained from Cuban, Puerto Rican and Mexican pineapples exhibited the same degree of anthelmintic activity *in vitro*, when tests were performed on *Ascaris* worms.

Action of Canned Juice.—*Ascaris* worms incubated at 37° C. with canned pineapple juice were not digested.

DISCUSSION

The fact that pineapple juice has been found to digest intestinal parasites *in vitro* gives some scientific support to the use of this juice as an anthelmintic by the native population of Brazil and India.

It has been experimentally demonstrated that the latexes of some species of Ficus (19), (20), (21) are very effective anthelmintics *in vitro* as well as *in vivo*. Their anthelmintic activity is due to the enzyme ficin which has been recently obtained in the crystalline form (22).

As the pineapple juice acts, *in vitro*, on parasites, in a fashion similar to the latexes, there is a probability that it may have the same effect *in vivo*. However, not until experiments under controlled conditions are performed with animals and human beings can this question be answered definitely.

SUMMARY

1. Fresh pineapple juice digests parasites *in vitro*. The time required for visible signs of digestion to take place is from three to four hours. Digestion takes place on the live parasite.

2. The juice maintains its digestive activity on *Ascaris* at a concentration of 15%.

3. A temperature of 65° C. or above inactivates the enzyme present in the juice.

4. Juice from Cuban, Puerto Rican and Mexican pineapples exhibits the same digestive activity on parasites *in vitro*.

5. Canned juice has no digestive activity on parasites.

REFERENCES

(1) Berger, J., and Asenjo, C. F., *Science*, 90, No. 2335, 299 (1939).

(2) Marcano, V., *Apoth. Ztg.* (1891), quoted in *Polytechn. Notizbl.*, 46, 159, Frankfurt (1891).

(3) Anonymous, *Pharm. Zentralhalle*, 32, 230 (1891).

(4) Anonymous, *Ibid.*, 32, 682 (1891).

(5) Anonymous, *Reichs.-Med.-Anz.*, 153 (1891); through reference No. 4.

(6) Anonymous, *Bull. of Pharm.*, 77 (1891).

(7) Chittenden, R. H., *Trans. Conn. Acad. Sc.*, 8, 281 (1891).

(8) Chittenden, R. H., *J. Physiol.*, 15, 249 (1893).

(9) Vines, S. H., *Annals of Botany*, XIX, 177 and 184 (1905).

(10) Caldwell, J. S., *The Botanical Gazette*, XXXIX, 409 (1905).

(11) Willstätter, R., Grassmann, W., and Ambros, O., *Z. Physiol. Chem.*, 151, 286 (1926).

(12) Ambros, O., and Harteneck, A., *Ibid.*, 181, 24 (1929); 184, 93 (1929).

(13) Tanaka, S., *J. Agr. Chem. Soc. Japan*, 454–455 (1930).

(14) Maschmann, E., *Z. Physiol. Chem.*, 228, 141 (1934).

(15) Bergmann, M., Fruton, J. S., and Fraenkel-Conrat, H., *J. Biol. Chem.*, 119, 35 (1937).

(16) Bergmann, M., and Fraenkel-Conrat, H., *Ibid.*, 119, 707 (1937).

(17) Peckolt, T., and Peckolt, G., *Historia das Plantas Medicinaes e Uteis do Brazil*, I, 178, Rio de Janeiro (1888).

(18) Nadkarni, K. M., *The Indian Materia Medica*, 62, Bombay (1927).

(19) Robbins, B. H., *J. Biol. Chem.*, 87, 251 (1930).

(20) Robbins, B. H., and Lamson, P. D., *Ibid.*, 106, 725 (1934).

(21) Caldwell, F. C., and Caldwell, F. L., *Am. J. Trop. Med.*, 9, 471 (1929).

(22) Walti, A. J., *J. Biol. Chem.*, 119, *Sc. Proc. Soc. Biol. Chem.*, xxxi, CI (1937).

A Pharmacologic Study of Devil's Club Root (*Fatsia Horrida*)*

By Leonard J. Piccoli, Ph.D., Michael E. Spinapolice, B.S., and Morris Hecht, B.S.

The treatment of diabetes by insulin injections has many obvious disadvantages. In order to obviate these factors many investigations have been made with oral preparations (1). Much controversy exists over the efficacy of these products (2). Recently, interest has been shown in the effects of aqueous extracts of Devil's Club Root (*Fatsia Horrida*). This report presents observations on the blood sugar metabolism of rabbits treated with these extracts.

EXPERIMENTAL

The dried root bark was ground to a coarse powder and extracted according to the method of Large and Brocklesby (3). The powder was boiled 3 times with twice its volume of water and the extract filtered off. Tannins were removed with neutral lead acetate and excess lead by means of H_2S. The precipitated tannins were separated by means of a Sharple's supercentrifuge. The excess H_2S was removed by boiling and the extract finally evaporated, so that the number of cc. of final volume was 1/3 the number of grams of the original bark. The extract was made slightly alkaline with magnesium oxide, filtered and stored for use. This type of extract was used throughout the investigation. The extract was then administered in varying doses to rabbits. In order to ensure complete administration of the doses, they were passed directly into the stomach by means of a catheter.

TABLE I.—GLUCOSE VALUE (MG. PER 100 ML. OF BLOOD) FROM UNMEDICATED RABBITS

Rabbit	Weight of Rabbit	Number of Readings	Low Reading	High Reading	Average	Average Deviations
A	4 lbs. 4 oz.	6	105.2	123.6	114.3	6.7
B	4 lbs. 8 oz.	6	98.5	132.4	116.5	7.2
C	6 lbs. 10 oz.	5	93.5	134.2	116.0	9.6
D	7 lbs. 4 oz.	7	90.7	129.9	113.7	7.9
E	8 lbs.	7	99.6	145.6	122.6	15.5

TABLE II.—GLUCOSE VALUES (MG. PER 100 ML. BLOOD) OBTAINED FROM RABBITS MEDICATED WITH DEVIL'S CLUB ROOT EXTRACT

Rabbit	Hours of Starvation	Extract[a] 1 Cc. = 3 Gm. of Drug	Dose per Lb. of Body Weight	Blood Sugar Value before Medication	Blood Sugar Values after Medication (Time in Hours) 1/3	1	1 1/3	2	3	4	5	6
	15	No. 2	0.14 cc.	107.4	...	131.6	...	99.0	120.0	119.3	...	...
A	15	No. 3	0.14 cc.	117.2	...	94.5	...	104.7	117.9	118.6	...	...
	24	No. 3	0.07 cc.	105.2	...	95.8	...	123.6	118.6	...	...	...
	15	No. 2	0.14 cc.	118.6	...	114.6	...	100.5	115.3	117.3	...	...
B	15	No. 3	0.14 cc.	132.4	...	103.5	...	109.0	123.6	113.9	...	...
	24	No. 3	0.07 cc.	98.5	...	107.4	...	117.2	110.9	...	...	...
	15	No. 2	0.14 cc.	116.0[b]	...	101.0	...	98.0	103.5	109.0	...	...
C	15	No. 3	0.14 cc.	120.7	...	108.5	...	106.8	103.5	105.2	...	...
	24	No. 3	0.14 cc.	93.5	...	95.8	...	115.3	109.7	...	...	...
D	16	No. 1	0.06 cc.	119.3	138.7	138.7	124.3	123.6	...	...	...	...
	16	No. 1	0.14 cc.	113.7[b]	...	...	147.1	...	173.8	...	147.1	145.7
E	16	No. 1	0.06 cc.	116.6	125.2	147.8	114.6	127.5	...	...	...	...
	16	No. 1	0.14 cc.	122.6[b]	...	...	141.6	...	126.5	...	113.2	127.5

[a] Three different extracts were prepared.

[b] The blood sugar values before medication were not taken. The figures are the averages obtained from Table I.

Normal rabbits weighing 4 1/4 to 8 lbs. were used. Blood was taken from the marginal ear vein and tested for glucose content by Benedict's modification of the Folin-Wu micro method (4). Normal blood sugar values for each rabbit are shown in Table I. It is to be noted that in all cases the blood sugar value never fell below 90 mg. of glucose per 100 ml. of blood, and the general average of the group was about 115 mg./100 ml.

Medication was started after having established these normals. The rabbits were starved for 15 to 24 hours before administration of the extract and blood sugar determinations were made at various

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intervals following ingestion. The results obtained are shown in Table II.

SUMMARY

1. Rabbit *A* showed a lowest blood sugar value of 94.5 mg./100 ml. and a highest value of 131.6 mg./100 ml., using 2 different doses and two different extracts. These figures are within the range of normal blood sugar values for *A* as indicated in Table I.

2. Similarly the lowest and highest values obtained for rabbits *B*, *C* and *E* also fall within the normal values for each.

3. Rabbit *D*, although showing values greater than the normal, did not give any below the normal.

CONCLUSION

These observations seem to indicate that oral administrations of our aqueous extracts of Devil's Club Root have no hypoglycemic effect upon the blood of rabbits which have been starved for 15 to 24 hours.

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REFERENCES

(1) Bischoff, F., Long, M. L., and Sahyun, *J. Pharmacol.*, 36, 311 (1929). Braun, C. E., and Rees, F. M., *J. Chem. Ed.*, 12, 453 (1935). Kelly, H. P., and Griffiths, C., *British Med. J.*, I, 921 (1925). Lasch, F., *Arch. Exptl. Path. Pharm.*, 122, 286 (1927). Lasch, F., and Brugel, S., *Ibid.*, 120, 144 (1927). Lasch, F., and Schonbrunner, E., *Klin. Wochschr.*, 17, 114 (1938); 17, 1177 (1938). Merlin, J. R., U. S. Pat. 1,646,553, Oct. 25 (1927). Mukerjee, H. N., *J. Physiol.*, 70, 182 (1930); 84, 362 (1935); *Biochem. J.*, 30, 1583 (1936). Steinitz, E., and Thau, Erno, *Arch. Verdauungs-Krankh.*, 49, 201 (1931). Walden, G. B., Can. Pat. 283,631, Sept. 25 (1928).

(2) "Herbal Treatment of Diabetes," *J. A. M. A.*, 111, 2395 (1928).

(3) Large, R. G., and Brocklesby, N. H., *Can. Med. Assoc. J.*, 39, 32 (1938).

(4) Peters, J. P., and Van Slyke, D. D., "Quantitative Clinical Chemistry," Vol. II, Methods, pages 456–460. The Williams and Wilkins Co., Baltimore, 1932.

The Constitution of Celastrol —Part II

*By O. Gisvold**

A preliminary report on the constitution of celastrol, a pigment obtained from *Celastrus scandens*, was given in a previous paper (1). Celastrol crystallizes from organic solvents in the form of ruby-red cubes. The following derivatives were reported: methyl celastrol (orange needles), acetyl celastrol (yellow), methyl acetyl celastrol (yellow) and acetyl methyl celastrol (yellow). Of the derivatives prepared, the methylated compound (prepared by methylation with diazomethane) was the only one to be obtained in a good crystalline form. Its methoxyl content was 9.01 (av.) which indicated the introduction of one methoxyl group. The molecular weight by the Rast method was 347. Celastrol and methyl celastrol gave a green color with ferric chloride whereas the acetylated derivatives did not. Celastrol appeared to be reversibly reduced at 190° C. with Raney's nickel. Ultimate analysis of celastrol indicated the formula $C_{23}H_{36}O_3$. It will be shown in this paper that this formula is not correct because not all the solvent of crystallization could be removed by drying at 100° C. over phosphorus pentoxide in a vacuum. Ultimate analysis of the acetate indicated the formula $C_{28}H_{34}O_5$.

Subsequent investigations on the constitution of celastrol indicate that no carboxyl groups are present in the molecule. In addition to the introduction of one methoxyl group, it will be shown that two acetyl groups can be introduced, thus accounting for the presence of three oxygens. One or both of the acetyl groups must be hydrolyzed very rapidly because alcoholic alkali causes an immediate development of a deep red color. It appears that a second methoxyl group can be introduced into the molecule by means of dimethyl sulfate and alkali. This dimethoxyl compound is yellow in color. Catalytic reduction of celastrol and methyl celastrol with platinum black at 40-lbs.

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pressure and ordinary temperatures gives colorless or nearly colorless products that give a green color with ferric chloride and are oxidized by atmospheric oxygen to red compounds again. Celastrol is unstable in the presence of potassium hydroxide and air. It is oxidized instantly to a pale yellow compound by means of hydrogen peroxide and alkali and is reversibly reduced by means of sulfurous acid.

The results of investigations to date indicate that celastrol has the formula $C_{22}H_{30}O_3$. One hydroxyl group is acidic enough to be methylated by means of diazomethane. The remaining two oxygen atoms appear to be in the form of an ortho quinone. One or both carbonyl groups evidently can be forced into an enolic form by acetylation as evidenced by the mitigation of color.

EXPERIMENTAL

Methyl Celastrol.—Because this derivative could be obtained in such an excellent crystalline state and because it appeared to have no solvent of crystallization, its ultimate analysis was highly desirable.

Analysis:

$C_{23}H_{32}O_3$	Calculated	C 77.56	H 8.99	
	Found	C 77.67,	H 8.97,	9.13,
		77.83	8.96	
		77.51		

Celastrol.—The values for carbon and hydrogen reported in the previous paper would not agree with the formula $C_{22}H_{30}O_3$.

It had been noted that celastrol appeared to undergo decomposition at the melting point with the evolution of a gas. In order to determine the nature of this gas, an experiment was devised whereby dry nitrogen was passed over a sample of celastrol (previously dried at 100° C. in a vacuum) at the melting point. The gases were then passed through dryrite and ascarite in order to absorb any water or carbon dioxide that might be evolved. No carbon dioxide or water was given off but the original compound lost weight. This was calculated as solvent (isopropyl ether) still retained by celastrol. A 0.2034-Gm. sample lost 0.0144 Gm., or 7.08 per cent, equal to one-fourth molecule of isopropyl ether. Therefore one molecule of isopropyl ether was present to four molecules of celastrol.

Analysis:

$(C_{22}H_{30}O_3)_4.C_6H_{14}O$	Calculated	C 76.73	H 9.11
	Found	C 76.66,	H 9.08,
		76.59	8.65

Reduction of Celastrol.—A qualitative catalytic reduction of celastrol by means of platinum black at room temperature and 40-lbs. pressure produced a colorless solution. The catalyst was removed by filtration and before it could be completed the solution became colored yellow and upon standing soon turned orange-red.

Reduction of Methyl Celastrol.—The reduction was carried out in the same manner described above. The same phenomenon took place. A portion of the colorless solution was tested with ferric chloride before oxidation could take place and an intense green color developed instantly.

Oxidation of Methyl Celastrol with Hydrogen Peroxide.—One-half gram of methyl celastrol was dissolved in an excess of alcoholic potassium hydroxide. Hydrogen peroxide (30%) was added until the deep red color was completely destroyed. This reaction took place very rapidly. The reaction mixture was acidulated with phosphoric acid and extracted with ether. The ethereal solution in turn was extracted with aqueous sodium bicarbonate. The combined bicarbonate extracts were washed once with ether, decomposed with phosphoric acid and extracted with ether. The ethereal extract was washed with water and the ether concentrated. About one-fourth Gm. of oxidized product was obtained which corresponded to one-half of the starting material. This oxidized material has not been obtained in a crystalline form as yet, but it appears to be acidic.

Reduction of Celastrol and Methyl Celastrol with Sulfurous Acid.—When an alcoholic solution of celastrol was treated with sulfurous acid the mixture immediately became colorless. Upon removal of the sulfurous acid whether by aeration or neutralization with alkali, the solution become colored again. Ortho quinones can be reduced by sulfurous acid.

When methyl celastrol was treated in the same fashion, the same phenomenon was observed.

Saponification of Acetyl Methyl Celastrol.—When potassium hydroxide was added to an alcoholic solution of acetyl methyl celastrol, the solution immediately turned dark red. If sodium carbonate was substituted for potassium hydroxide no immediate red color appeared. However, a red color soon developed which increased in intensity from a very pale red to a deep garnet-red. The mixture was then diluted with water and extracted with ether. The ether was removed on the steam-bath and the residue taken up in alcohol. The alcoholic solution gave a green color with ferric chloride T.S.

This experiment indicates that the acetyl groups are extremely sensitive to alkaline hydrolysis.

Methylation of Methyl Celastrol by Dimethyl Sulfate and Alkali.—Methyl celastrol was methylated in the usual manner with dimethyl sulfate and alkali. Because methyl celastrol and celastrol are unstable in the presence of alkali, it is doubtful if a uniform product was obtained. This methylated product was yellow and could not be crystallized. The methoxyl content indicated the equivalent of almost two methoxyl groups.

Analysis—OCH_3:

$C_{24}H_{24}O_3$ Calculated for 2 methoxyl groups 16.75 per cent
Found 16.15, 16.00; av. 16.08 per cent

Acetyl Value of Acetyl Celastrol.—Acetyl celastrol was saponified with an excess of alcoholic alkali. The reaction mixture was acidulated with sulfuric acid and the liberated acetic acid distilled into standard alkali.

Analysis—CH_3CO:

$C_{28}H_{34}O_8$ Calculated for 2 acetyl groups 20.14 per cent
Found 21.44, 21.75; av. 21.59 per cent

Acetyl Value of Acetylated Methylated Celastrol.—This analysis was carried out like the above analysis.

Analysis—CH_3CO:

$C_{27}H_{36}O_8$ Calculated for 2 acetyl groups 19.50 per cent
Found 19.82, 20.69; av. 20.25 per cent

Attempted Reduction of Methyl Celastrol with Aluminum Isopropylate.—Aluminum isopropylate was prepared according to the method of Homer Adkins (2). A test reduction of methyl celastrol with aluminum isopropylate indicated that an aluminum salt of methyl celastrol was formed, because the mixture assumed a deep red color. The mixture when heated for ten hours did not change in color intensity and no reduction took place. According to Lund (3), ketones which tend to enolize and form aluminum salts cannot be reduced by this method.

SUMMARY

It has been shown that celastrol, a red pigment found in the outer bark of the root of *Celastrus scandens*, has the formula $C_{22}H_{30}O_3$. The molecule contains an hydroxyl group sufficiently acidic enough to be methylated by means of diazomethane. It appears that the remaining two oxygen atoms are present in the form of an ortho quinone which can be reduced catalytically and reoxidized by atmospheric oxygen.

REFERENCES

(1) Gisvold, O., JOUR. A. PH. A., 28, 441 (1939).
(2) Adkins, H., *J. Am. Chem. Soc.*, 44, 2175 (1922).
(3) Lund, H., *Ber.*, 70B, 1520 (1937); *C. A.*, 31, 6611 (1937).

Water in Oil Emulsifying Agents. I*

Some Constituents of Lanolin and Similar Compounds

By J. L. Powers,† H. B. Leask† and R. S. Warner††

Water-in-oil emulsifying agents are used extensively in the manufacture of ointments and cosmetics. Among the more widely used emulsifying agents for these purposes are waxes such as lanolin spermaceti, and beeswax; polycyclic alcohols such as sterols; and aliphatic alcohols of high molecular weight, some of which are components of certain waxes. Of these, lanolin and waxes were used by the ancients (1). More recently ointment bases of high water absorptive power have been introduced, and are usually described in rather vague terms as consisting of certain alcoholic components of lanolin combined with petrolatum (2). Several patents have been issued on methods of obtaining components of lanolin with unusual hydrophylic properties, of which those granted to Lifschütz (3), (4), Frick (5) and Barnes (6) are examples. Cholesterol has been recommended frequently as a useful adjunct to petrolatum for increasing its ability to absorb water, and for enhancing its value from a therapeutic or cosmetic standpoint (7), (8), (9), (10), (11), (12), (13). Blatz (14), some years ago, showed that cetyl alcohol when mixed with petrolatum produced an ointment base capable of absorbing 60% to 70% of water.

The earlier investigations of the chemical components of lanolin by Schulze (15), (16) and by Darmstaedter and Lifschütz (17) indicated that the unsaponifiable portion consisted principally of cholesterol, iso-cholesterol and aliphatic alcohols such as ceryl alcohol. More recently iso-cholesterol has been resolved into agnosterol (18), $C_{30}H_{48}O$, and lanosterol (19), $C_{30}H_{50}O$. The investigations of Schulze (20) and others (21), (22)

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indicate that neither agnosterol nor lanosterol is a true sterol. Sobotka (23) states, "They have thirty carbon atoms and a picene skeleton, consisting of five six-membered rings with six non-nuclear methyl groups. On dehydrogenation, they never yield methyl-CPP,[1] but picene and a number of substituted naphthalenes."

Lifschütz (24) has shown, in a semi-quantitative way, the influence of certain alcoholic components of lanolin as water-in-oil emulsifying agents when used with petrolatum. Lifschütz (25) has also pointed out that lanolin as an ointment base possesses chemical and physical disadvantages, and that its water-absorptive property is due to the presence of from 5% to 8% of free alcohols. He recommends a mixture of 3% of these alcohols with petrolatum as an ointment base which will absorb from 300% to 400% of water. Casparis and Meyer (26) have determined the maximum amount of water which mixtures of several different waxes and alcohols with petrolatum and lard are capable of absorbing. The maximum quantity of water capable of being held at ordinary temperatures (20° C.) by 100 Gm. of water-free ointment base, they designate as "water number."

Despite the extensive literature relating to lanolin and its use and the use of its components in hydrophylic ointment bases, some confusion continues to exist. Accordingly, an investigation was undertaken to ascertain with some degree of certainty what components of lanolin are responsible for its ability to absorb water, and, if possible, to develop an easily obtainable hydrophylic emulsifier which when mixed with petrolatum would produce a water-absorbent ointment base for general use, but without the inherent objectionable features of lanolin when used alone.

Anhydrous lanolin was saponified by three different methods: (1) that of Doree and Garratt (19) involving the use of sodium ethylate in anhydrous ether; (2) heating the lanolin with alcoholic potassium hydroxide under a reflux condenser during twelve hours; and (3) by a cold method not previously described for the saponification of lanolin. The latter method appeared to be the most convenient and quite satisfactory since it yielded as good results as the first method. The unsaponifiable portion was separated from the soaps and resolved into four fractions, the first, consisting of a mixture of aliphatic alcohols, the second, of the so-called "iso-cholesterol," the third, of cholesterol, while the fourth fraction was an amorphous mixture from which no crystalline material could be isolated. The partial separation of the unsaponifiable portion was accomplished by modifications of the methods described by Doree and Garratt (19), Heiduschka and Nier (27) and Kuwata and Katuno (28). Mixed amyrins were obtained from Manila Elemi Gum, and resolved into α- and β-forms through the benzoates by the method of Horrmann (29). Cholesterol esters of lauric, palmitic and stearic acids were prepared by the procedures described by Page and Rudy (30). The cetyl alcohol used was obtained from the Eastman Kodak Company. The water-absorbent powers of these various substances in different concentrations in white petrolatum[2] were determined. Amyrin was included in the list because of the possibility that it might develop that there is a close chemical relationship between it and "iso-cholesterol."

[1] Cyclo-penteno-phenanthrene.

EXPERIMENTAL

Saponification of Lanolin.—To 800 Gm. of melted anhydrous lanolin (Merck) were added 200 cc. of alcohol, and the mixture was cooled to 34°. To this mixture were added with vigorous stirring 270 Gm. of potassium hydroxide dissolved in 300 cc. of water, and cooled to room temperature. When the reaction had subsided, the mixture was placed in an oven at 50°, and dried for twenty-four hours. It was then mixed with 600 Gm. of anhydrous sodium sulfate, transferred to a Soxhlet extractor, and extracted with acetone until all of the unsaponifiable portion and a small amount of the soap were removed. The acetone solution was concentrated to a volume of 500 cc., one L. of ether was added, and the solution was washed with water in a separatory

[2] The white petrolatum which appeared to give best results was White Protopet No. 1 produced by L. Sonneborn and Sons. We wish to thank Dr. Erich Meyer for suggesting its use, and for supplying us with an adequate quantity for our experiments.

funnel several times. In this way all soaps were removed without the formation of an emulsion. Upon evaporation of the solvent, a residue weighing 300 Gm. was obtained.

Resolution of the Unsaponifiable Portion.—One hundred Gm. of the unsaponifiable portion of lanolin were dissolved in hot acetone. The solution, upon cooling, deposited 17 Gm. of nearly white amorphous material which after one recrystallization from methyl alcohol, followed by one recrystallization from acetone, yielded 9 Gm. of crystalline material melting at 58–59°, with preliminary softening. On the basis of work previously referred to (19), (27), (28), this was considered to consist of a mixture of aliphatic alcohols. No attempt to make a further separation of this first fraction was successful.

The acetone (675 cc.) was removed by distillation from the solution remaining after the separation of the mixture of aliphatic alcohols. The residue was extracted repeatedly with hot methyl alcohol until a white, pulverulent residue weighing 9.5 Gm. remained. This material, after one recrystallization from a mixture of acetone and methyl alcohol, and drying at 100°, melted at 135–136° with preliminary softening. This is near the melting point given in the literature for iso-cholesterol, and since no precipitation was produced from an alcoholic solution of this second fraction when an alcoholic solution of digitonin was added, it was considered to be sufficiently pure for testing its emulsifying power for water when mixed with petrolatum.

The methyl alcoholic solution obtained from the preceding extraction was cooled to 15°, whereupon 18 Gm. of nearly white, crystalline material were obtained. This was recrystallized once from methyl alcohol, and then acetylated by means of acetic anhydride. The mixture of acetates, recrystallized several times from 95% alcohol, and finally from acetone, yielded a small fraction (2.2 Gm.) melting at 112–114°. When this was mixed with cholesteryl acetate, there was no depression in melting point. Upon saponification, and recrystallization of the product from 95% alcohol, cholesterol melting at 146–147° was obtained. Mixed m. p., 145–146°. Since appreciable quantities of pure cholesterol could not be obtained from this third portion by fractional crystallization, commercial cholesterol (Eastman's) was used in the water absorption experiments.

The methyl alcoholic solution, after removal of the 18 Gm. of crystalline material, was concentrated in volume, and an additional 20 Gm. of yellowish white, amorphous material was obtained. This, after recrystallization from acetone, melted at 55–60°. No attempt to make a further separation by fractional crystallization was successful. Finally, upon removal of all of the methyl alcohol, 33 Gm. of an amorphous, gummy residue remained. No crystalline material could be obtained from this fourth fraction, and it remained apparently unaltered when boiled during several hours with 15% alcoholic potassium hydroxide.

Preparation of Cholesteryl Laurate.—Four Gm. of cholesterol (Eastman's, m. p. 145–146°) and 8 Gm. of lauric acid (Eastman's, m. p. 42–43°) were thoroughly mixed. The mixture was heated during 3 hours in an atmosphere of carbon dioxide at a temperature of 200°. The product, recrystallized three times from alcohol, yielded 5.5 Gm. of the ester melting at 91° (30).

Preparation of Cholesteryl Palmitate.—Five Gm. of cholesterol and 6 Gm. of palmitic acid (Eastman's, m. p. 61–62°) were thoroughly mixed, and then heated during two hours in an atmosphere of carbon dioxide at a temperature of 210–220°. After recrystallizing the product four times from alcohol, 6 Gm. of the ester melting at 89° with preliminary softening were obtained (30).

TABULATION OF EMULSIFYING POWER OF SOME WATER IN OIL EMULSIFYING AGENTS WITH PETROLATUM

Emulsifying Agent in White Petrolatum	Per Cent of Water Emulsified
Cholesterol 3%	250%
Cholesterol 3% + Cholesteryl Acetate 3%[a]	500%
Cholesterol 3% + Cholesteryl Laurate 3%[a]	600%
Cholesterol 3% + Cholesteryl Palmitate 3%[a]	700%
Cholesterol 3% + Cholesteryl Stearate 3%[a]	800%
Cholesterol 3% + Spermaceti 3%	500%
Cholesterol 3% + White Wax 3%	500%
Spermaceti 3%	100%
Iso-Cholesterol 3%[b]	300%
α-Amyrin 2%[b]	50%
β-Amyrin 2%[b]	50%
Mixture of Aliphatic Alcohols (Fract. I) 3%[c]	400%
Cetyl Alcohol 3%[c]	75%
Total Unsaponifiable from Lanolin 5%	350%
Unsaponifiable 4% + White Wax 4%	600%
Fract. 4 from Unsaponifiable 3%	400%

[a] Cholesterol esters when used alone have no emulsifying action.

[b] The efficiency of the amyrins and iso-cholesterol is not appreciably increased when combined with their esters or with waxes.

[c] It is not possible to reconcile these different results unless it is assumed that they are due to the longer carbon chain in ceryl alcohol. The efficiency of cetyl alcohol is not materially increased by mixing it with small amounts of cholesterol or iso-cholesterol.

Preparation of Cholesteryl Stearate.—Four Gm. of cholesterol were thoroughly mixed with 6 Gm. of stearic acid (Eastman's, m. p. 69–70°). The mixture was heated during three hours in an atmosphere

of carbon dioxide at a temperature of 210–220°. The product was recrystallized twice from a mixture of equal volumes of acetone and methyl ethyl ketone. It then was dissolved in 50 cc. of ether, and precipitated by the addition of 200 cc. of alcohol. Yield, 6.1 Gm. Melting point, 82° with preliminary softening (30).

Determination of Emulsifying Power.—Into each of five numbered 8″ x 1″ test-tubes were placed 5 Gm. of a 1% solution of cholesterol in white petrolatum. The first test-tube was immersed in a bath of boiling water, and heated until the solution reached a temperature of 65–70°. To this were added all at one time, with mechanical stirring by means of a spiral glass stirrer reaching to the bottom of the test-tube, 2.5 cc. of water at the same temperature. The mixture was stirred until it had cooled to room temperature. Under identical conditions, 5 cc. of water were added to the second test-tube, 7.5 cc. to the third, 10 cc. to the fourth and 12.5 cc. to the fifth. Similarly, varying proportions of water were added to 2, 3, 4 and 5% solutions of cholesterol in white petrolatum. The emulsions thus formed were set aside for two months, and then examined. A decision was then reached as to the optimum concentration of cholesterol in petrolatum for the production of an emulsion containing a maximum amount of water. By identical methods the other emulsifying agents were similarly studied. In the tabulation which follows are reported the concentration and composition of the various emulsifying agents which were believed to be best for producing stable emulsions containing the highest concentrations of water.

SUMMARY AND CONCLUSIONS

1. Lanolin has been saponified and the unsaponifiable portion has been separated into four fractions.

2. The relative efficiencies of certain components of lanolin and some analogous compounds as water-in-oil-emulsifying agents with petrolatum have been determined.

3. It has been shown that the emulsifying efficiency of cholesterol is much less than the emulsifying efficiency of mixtures of cholesterol and cholesterol esters when used with petrolatum for the purpose of emulsifying water.

4. Any one combination of petrolatum and water-in-oil emulsifying agent as a suitable ointment base cannot be recommended until after further study.

REFERENCES

(1) Schelenz, H., "Geschichte der Pharmazie," Springer, Berlin, 105 (1904).

(2) Unna, E., *Chem. Abstracts*, 7, 864 (1913); 8, 1186 (1914).

(3) Lifschütz, I., *Friedlaender*, 13, 923 (1916–1921).

(4) Lifschütz, I., U. S. Pat. 1,251,621 (1918).

(5) Frick, F., Ger. 485,198 (1926); *Chem. Abstracts*, 24, 983 (1930).

(6) Barnes, B., U. S. Pat. 1,830,502 (1931).

(7) Siedler, P., *Apoth. Zeit.*, 27, 211 (1912).

(8) Rapp, A., *Pharm. Zeit.*, 75, 303 (1930).

(9) Schmatolla, O., *Ibid.*, 77, 727 (1932).

(10) Ruemele, T., *Deut. Parfümerie Zeit.*, 18, 86 (1932); *Chem. Abstracts*, 26, 5175 (1932).

(11) Kramer, R., *Drug and Cosmetic Ind.*, 37, 741 (1935).

(12) Schwartz, H., *Seifensieder-Zeit.*, 63, 170 (1936); *Chem. Abstracts*, 30, 3165 (1936).

(13) Ping-Lu Li, and Kuever, R., *J. Am. Pharm. Assoc.*, 27, 1217 (1938).

(14) Blatz, F., *Apoth. Zeit.*, 24, 523 (1909).

(15) Schulze, E., *Ber.*, 5, 1075 (1872); 6, 251 (1873); 7, 570 (1874).

(16) Schulze, E., *J. prakt. Chem.*, 7, 163 (1873); 9, 321 (1874).

(17) Darmstaedter, L., and Lifschütz, J., *Ber.*, 28, 3133 (1895); 29, 618, 1474 (1896); 31, 97, 1122 (1898).

(18) Windaus, A., and Tschesche, R., *Z. physiol. Chem.*, 190, 51 (1930).

(19) Doree, C., and Garratt, D., *J. Soc. Chem. Ind.*, 52, 141T, 355T, (1933).

(20) Schulze, H., *Z. physiol. Chem.*, 238, 35 (1836).

(21) Marker, R., Wittle, E., and Mixon, L., *J. Am. Chem. Soc.*, 59, 1368 (1937).

(22) Marker, R., and Wittle, E., *Ibid.*, 59, 2289 (1937).

(23) Sobotka, H., "Chemistry of the Sterids," Williams and Wilkins, Baltimore, 79 (1938).

(24) Lifschütz, I., *Z. physiol. Chem.*, 114, 108 (1921).

(25) Lifschütz, I., *Pharm. Zentralhalle*, 63, 305 (1923).

(26) Casparis, P., and Meyer, E., *Pharm. Acta Helv.*, 10, 163 (1935).

(27) Heiduschka, A., and Nier, E., *J. prakt. Chem.*, 149, 98 (1937).

(28) Kuwata, T., and Katuno, M., *J. Soc. Chem. Ind. Jap.*, 41, 227 (1938); *Chem. Abstracts*, 32, 9536 (1938).

(29) Horrmann, P., and Martinius, J., *Arch. Pharm.*, 268, 64 (1930).

(30) Page, I., and Rudy, H., *Biochem. Z.*, 220, 304 (1930).

Hydrogenated Oil as an Ointment Base. II*

George W. Fiero†

Several years ago the writer reported that hydrogenated cottonseed oil was satisfactory as an ointment base to replace lard (1). At the present time hydrogenated peanut oil is the official base for several ointments in the Swiss Pharmacopœia (2), and other hydrogenated oils are being employed commercially in the United States in proprietary ointments. Hydrogenated castor oil was found to have characteristics which made it of value for ointments (3), particularly emulsified creams and cosmetics (4). It is the purpose of the present study to compare those hydrogenated oils which are being manufactured commercially at the present time.

Some hydrogenated oils (shortenings) in reality are merely the unhydrogenated oil with sufficient completely hydrogenated oil to give the desired consistency. Obviously, this is more economical, but from the standpoint of rancidity these oils are much more susceptible to deterioration than oils which had been partially hydrogenated. The principal advantage of hydrogenated oil over lard as an ointment base is the fact that, because of partial reduction, it is less susceptible to rancidity, and ointments prepared with it are more stable.

The hydrogenated oils used in this experiment are listed together with certain characteristics in Table I. The writer wishes to acknowledge the coöperation of the following manufacturers for samples and data (except softening point) in this table: Armour and Co., Chicago, Ill.; The Cudahy Packing Co., Chicago, Ill.; Durkee Famous Foods, Elmhurst, N. Y.; Mefford Chemical Co., Los Angeles, Calif.; The Proctor and Gamble Co., Ivorydale, O.; The Southern Cotton Oil Co., Savannah, Ga.; Spencer Kellogg and Sons, Inc., Buffalo, N. Y.; Swift and Co., Chicago, Ill.; Vegetable Oil Products Co., Inc., Wilmington, Calif., and Wilson and Co., Chicago, Ill.

EXPERIMENTAL

Melting points reported in Table I are not of uniform method. From the standpoint of ointments, it was considered that the softening point was more important; this was determined by the A. O. C. S. method (5).

Rancidity.—Samples of the various hydrogenated oils were melted and poured into test-tubes. One series was placed in a south window in direct sunlight (starting in February 1939) and the other series in the dark in an incubator at 36.5° C. From time to time the samples were examined and rancidity determined by the Kreis test (7). The results are indicated in Table II.

The data in Table II do not show a definite relationship between iodine number and rancidity; these samples were obtained from various manufacturers. In order to indicate the relationship between iodine value and rancidity, samples of the same batch of cottonseed oil were hydrogenated to various iodine values.[1] These were exposed to sunlight in the same manner as previous samples, differing, however, in the time of year, being exposed in May and June 1939. The results are shown in Table III.

Table II indicates that Hydrogenated Sesame Oil with an iodine value of 57.7 was by far the most stable of the partially hydrogenated oils. In order to determine if this is due to the relatively low iodine value, or to some inherent features of the oil, samples of the same batch of sesame oil were hydrogenated to various iodine values[1] and treated as above. The data are shown in Table III.

Consistency.—In order to make relative comparisons of the consistency, the hydrogenated oil was placed in a tin-coated, collapsible one-ounce tube with nasal tip and 4-mm. opening. The tubes were uniformly filled with the molten oil, sealed and allowed to cool. They were placed in an apparatus (see Fig. 1) consisting of a lever which compressed the tube while it was held in position by means of a perforated, beveled strip. The relative consistency was determined by the weight of material which would pass through the tip when various weights were added to the pan at the end of the lever for a definite length of time. The results are shown in Table IV.

Relative consistency was also determined by another method dependent upon the resistance of the fat to an object rather than a measure of the fluidity of the fat when compressed. The apparatus (Fig. 2) consisted of a metal disk 1.6 cm. in diameter attached to a 44-cm. vertical rod with a pan upon which weights could be placed. A scale was attached to the rod so that the extent of pene-

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[1] The writer is indebted to the Research Department of Armour and Co., Chicago, for preparing these samples.

tration could be measured. The melted fat was placed in a two-ounce jar and allowed to stand at room temperature for several days. The test was conducted by placing the disk below the surface of the fat (to avoid surface tension) and applying weights to the pan, measuring the extent of penetration after 60 seconds. The data are shown in Table V.

Absorption of Water.—Samples of various hydrogenated oils were added to an equal volume of water and allowed to stand with frequent agitation for 24 hours at a temperature above their melting points. They were then removed from the oven and allowed to stand at room temperature without agitation for 48 hours and then at 5° C for six days, as previously reported for hydrogenated castor oil (3). The fat was separated, adhering water removed and the moisture determined by the toluene-distillation method. The results are shown in Table VI.

TABLE I.—CHARACTERISTICS OF HYDROGENATED OILS USED

Hydrogenated Oil	Sample No.	Softening Point	Iodine Value	Melting Point	Free Fatty Acid (Oleic)	Saponification Value
Cottonseed, soft	1	36.3	66.7	38° C.	0.04%	199
Cottonseed, soft	2	37.2	62.0	45	0.02	197
Cottonseed, soft	3	39.4	66–9	39–40	0.02–0.04	...
Cottonseed, soft	4	29.5	...	38.0	0.01	...
Cottonseed, soft	5	31.3	66.0	...	0.02	196
Cottonseed, soft	6	35.7	62.0	37.2	0.01	...
Cottonseed, soft	7	41.5	62.7	43.3	0.02	...
Cottonseed, soft	8	29.8	70.0	39.0	0.03	198
Cottonseed, soft	9	41.8	57.9	44.0	...	...
Cottonseed, soft[a]	10	38.0	60.5	38.0	...	...
Cottonseed, soft[a]	11	37.7	65.0	41.0	0.03	...
Cottonseed, soft[a]	12	29.7	73.0	39.6	0.03	...
Cottonseed, hard	1	...	5.8	61.0	1.27	194
Cottonseed, hard	2	...	12.0	60.5	0.15	197
Cottonseed, hard	3	...	...	61.0	0.44	...
Cottonseed, hard	4	...	8.3	...	0.04	195
Cottonseed, hard	5	...	37.5	59.0	...	...
Cottonseed, hard	6	...	8.0	52.5	...	195
Untreated Cottonseed Oil, U. S. P.	..	...	105–114	...	...	190–198
Soybean, soft	1	36.8	70.3	39.4	0.04	192
Soybean, soft	2	36.5	72.0	42.0	0.03	192
Soybean, soft	3	35.9	72–6	38–9	0.02–0.04	...
Soybean, soft	4	35.8	74.0	...	0.02	195
Soybean, soft	5	40.8	83.0	46.0	0.05	190
Soybean, hard	1	...	4.4	62.5	0.53	192
Soybean, hard	2	...	9–16	61.0	0.02–0.04	...
Soybean, hard	3	...	10.0	65.5	...	190
Untreated Soybean Oil (6)	..	...	124–143	...	...	190.6–194.3
Coconut Oil	1	...	5.1	44.9	0.06	248
Coconut Oil	2	32.4	4–12	33.0	<0.035	252–260
Coconut Oil	3	43.0	4–12	43.0	<0.035	252–260
Coconut Oil[b]	4	34.4	1.5–3.5	38–9	0.01–0.02	...
Coconut Oil[c]	5	40.4	3.0	49.0	0.07	...
Untreated Coconut Oil (6)	..	...	8–9.6	23–26	...	251–263
Palm Kernel Oil	1	...	0.5–1	47–8	0.01–0.02	...
Palm Kernel Oil	2	...	0.5–1	48.5–9.5	0.01–0.02	...
Untreated Palm Kernel Oil (6)	..	...	...	16–23	24–30	244–255
Palm Oil	..	...	2.0	59.0	0.17	...
Untreated Palm Oil (6)	..	...	48–58	27–50	...	195–205
Peanut Oil, soft	..	40.7	73.3	42.5	0.04	190
Peanut Oil, hard	..	...	24.0	58.5	0.11	190
Untreated Peanut Oil (6)	..	...	83–95	...	...	185–190
Sesame Oil	..	42.7	57.7	45.6	0.04	189
Untreated Sesame Oil, N. F.	..	...	103–115	...	...	188–193
Lard, soft	1	38.0	51.0	44.9	0.06	194
Lard, soft	2	38.1	58–61	47–8.5	<0.03	192–6
Lard, hard	1	...	0.4	60.1	0.12	190
Lard, hard	2	...	< 2.0	61–2	<0.02	192–6
Untreated Lard U. S. P.	..	...	46–70	36–42	...	195–203

[a] A mixture, chiefly cottonseed oil. [b] With lecithin. [c] With a small amount of cottonseed oil.

TABLE II.—RANCIDITY OF HYDROGENATED OILS

Hydrogenated Oil	Sample No.	Iodine Value	Kreis Test 5 Weeks A	5 Weeks B	10 Weeks A	10 Weeks B	15 Weeks A	15 Weeks B
Cottonseed	1	66.7	0	0	4	0	7	0
Cottonseed	2	62.0	0	0	1	0	7	0
Cottonseed	3	66–9	1	0	2	0	7	0
Cottonseed	4	...	0	0	1	0	2	0
Cottonseed	6	62.0	T	0	2	0	7	0
Cottonseed	7	62.7	T	0	3	0	6	0
Cottonseed	8	70.0	0	0	2	0	7	0
Cottonseed	9	57.9	T	0	2	0	4	0
Cottonseed	10	60.5	1	0	3	0	6	0
Cottonseed	11	65.0	1	T	4	T	7	T
Cottonseed	12	73.0	T	T	2	T	7	T
Cottonseed, hard	1	5.8	1	0	1	T	4	T
Cottonseed, hard	5	37.5	2	0	4	0	6	T
Soybean	1	70.3	0	0	0	0	3	0
Soybean	2	72.0	T	0	3	0	6	0
Soybean	3	72–6	T	0	1	0	7	0
Soybean	5	83.0	T	0	2	0	7	0
Soybean, hard	2	9–16	0	0	T	0	4	T
Soybean, hard	4	...	0	0	T	0	T	0
Coconut	1	5.1	1	0	2	0	4	0
Coconut	2	4–12	1	0	2	0	7	0
Coconut	3	4–12	1	0	2	0	5	0
Coconut	4	1.5–3.5	2	0	5	0	7	0
Palm Kernel	1	0.5–1	.	.	.	.	.	.
Palm Kernel	2	0.5–1	T	.	1	T	1	T
Palm	..	2.0	T	T	T	T	T	T
Peanut, soft	..	73.3	1	0	5	0	7	0
Peanut, hard	..	24.0	T	0	T	0	T	0
Sesame	..	57.7	0	0	0	0	1	0
Lard, soft	1	51.0	2	1	3	2	7	4
Lard, soft	2	58–61	2	1	3	2	7	4
Lard, hard	1	0.4	T	0	1	0	1	0
Lard, hard	2	...	.	.	.	.	.	.
Control (untreated lard)	..	...	3	1	7	2	7	4

Legend: 0 = not rancid; T = trace of color; color of Kreis test indicated numbers varying from pink (1) to wine red (7).

TABLE III.—RANCIDITY IN OILS HYDROGENATED TO VARIOUS IODINE VALUES

Hydrogenated Oil	Iodine Value	Kreis Test 10 Days	20 Days	30 Days
Cottonseed Oil	74.9	1	3	5
Cottonseed Oil	70.7	1	3	5
Cottonseed Oil	65.9	0	1	3
Cottonseed Oil	59.5	0	0	1
Cottonseed Oil	53.3	0	0	1
Sesame Oil	78.9	0	T	1
Sesame Oil	73.3	0	0	T
Sesame Oil	69.8	0	0	0
Sesame Oil	65.5	0	0	0
Sesame Oil	61.5	0	0	0

TABLE IV.—CONSISTENCY OF HYDROGENATED OILS. GRAMS EXUDED BY COMPRESSION

Hydrogenated Oil	Sample No.	S. P.	0 Gm.	50 Gm.	100 Gm.	200 Gm.	300 Gm.	400 Gm.	500 Gm.	1000 Gm.	Total
Cottonseed	1	36.3	T	T	0.05	0.10	0.15	0.15	0.15	1.60	2.20
Cottonseed	2	37.2	0.10	0.15	0.20	0.25	0.30	0.50	0.80	5.50	7.80
Cottonseed	3	39.4	0	0	0	0	0	T	T	T	T
Cottonseed	4	29.5	0.25	0.45	0.50	0.80	0.85	0.80	0.90	1.15	5.70
Cottonseed	6	35.7	0	0	0	0	0	T	T	T	T

TABLE IV.—CONSISTENCY OF HYDROGENATED OILS. GRAMS EXUDED BY COMPRESSION

(*Continued from page 20.*)

Hydrogenated Oil	Sample No.	S. P.	0 Gm.	50 Gm.	100 Gm.	200 Gm.	300 Gm.	400 Gm.	500 Gm.	1000 Gm.	Total
Cottonseed	7	41.5	0	0	0	0	0	0	0	0	0
Cottonseed	8	29.8	0.25	0.30	0.40	0.80	0.80	0.80	0.85	1.25	5.45
Cottonseed	9	41.8	0	0	0	0	0	0	0	0	0
Cottonseed	10	38.5	0	0	0	0	T	T	T	T	T
Cottonseed	11	37.7	0.40	0.50	0.50	0.65	0.70	0.90	1.50	1.90	7.05
Cottonseed	12	29.7	0.80	1.05	1.30	1.80	1.10	0.65	0.35	1.30	9.35
Soy Bean	1	30.8	0	0	0	0	0	0	0	0	0
Soy Bean	2	36.5	1.20	1.40	1.60	2.30	1.60	1.30	1.30	5.20	14.30
Soy Bean	3	35.9	0	T	T	T	T	T	0.05	0.05	0.10
Soy Bean	5	40.8	0.05	0.05	0.05	0.05	0.10	0.10	0.10	6.50	7.00
Peanut	..	40.7	0	0.95	1.10	2.05	2.25	2.45	2.45	7.80	19.05
Lard	1	38.0	0	0	0	0	0	0	0	0	0
Lard	2	38.1	0	0	0	0	0	0	0	T	T
Coconut with lecithin	..	34.4	0.30	0.30	0.35	0.40	0.50	0.60	0.65	9.00	12.10
Petrolatum	..	..	0	T	T	0.05	0.10	0.15	0.20	1.35	1.85
Wool Fat	..	..	T	T	0.10	0.20	0.25	0.70	0.75	2.85	4.85
Lard	..	..	0.70	0.70	0.95	2.90	2.10	2.10	1.10	2.40	10.85

TABLE V.—CONSISTENCY OF HYDROGENATED OILS. EXTENT OF PENETRATION

Hydrogenated Oil	Sample No.	S. P.	100 Gm.	200 Gm.	300 Gm.	400 Gm.	500 Gm.	1000 Gm.	500[a] Gm.	1000[a] Gm.
Cottonseed	1	36.3	0.4	21.5	+	...	...	...	...	...
Cottonseed	2	37.2	2.0	4.0	+	...	...	...	...	...
Cottonseed	3	39.4	0.1	0.9	3.3	2.7	2.3	+	...	...
Cottonseed	4	29.5	1.0	1.0	1.5	2.5	+	...	...	...
Cottonseed	5	31.3	0.9	2.8	13.2	+	...	...	...	...
Cottonseed	6	35.7	–	–	–	–	–	2.5	3.0	+
Cottonseed	7	41.5	–	–	–	–	–	1.0	1.0	+
Cottonseed	8	29.8	1.5	7.5	9.0	9.0	8.0	+	...	...
Cottonseed	9	41.8	–	–	–	–	–	0.1	–	1
Cottonseed	10	38.0	5.5	7.0	8.0	4.0	2.5	+	...	...
Cottonseed	11	37.7	0.2	0.5	0.5	1.9	2.0	+	...	...
Cottonseed	12	29.9	+	...	...	...	...	...	...	...
Soy Bean	1	36.8	–	–	–	–	–	–	–	4
Soy Bean	2	36.5	2.25	+	...	...	...	...	...	...
Soy Bean	3	35.9	1.0	2.0	4.0	5.0	6.5	+	...	...
Soy Bean	4	35.8	0.1	0.1	0.1	0.4	0.4	...	...	...
Soy Bean	5	40.8	0.5	1.0	2.0	5.0	+	...	...	...
Peanut	..	40.7	+	...	...	...	...	...	...	...
Sesame	..	42.7	–	–	–	–	–	0.5	–	0.5
Lard	1	38.0	–	–	–	–	–	0.2	0.3	1.0
Lard	2	38.1	0.9	+	...	...	...	...	...	...
Coconut	..	32.4	–	–	–	–	–	–	–	2.0
Petrolatum		...	5	+	...	...	...	...	...	...
Wool Fat		...	19	+	...	...	...	...	...	...
Lard		...	9	+	...	...	...	...	...	..

Numbers indicate penetration in mm. after 60 seconds—using 1.6-cm. disk.

[+] Indicates rapid penetration to the bottom of the jar in less than 60 seconds.

[a] Mm. penetration after 60 seconds, using 1.1-cm. disk.

TABLE VI.—ABSORPTION OF WATER BY HYDROGENATED OILS

Hydrogenated Oil	Sample No.	Iodine Value	M. P.	Moisture Content %
Cottonseed, soft	2	62.0	45.0	2.00
Cottonseed, soft	3	66–9	39–40	4.35
Cottonseed, soft	4	...	38.0	0.50
Cottonseed, soft	6	62.0	37.2	3.00
Cottonseed, soft	7	62.7	43.3	4.50

TABLE VI.—ABSORPTION OF WATER BY HYDROGENATED OILS. (*Continued from page 21.*)

Hydrogenated Oil	Sample No.	Iodine Value	M. P.	Moisture Content %
Cottonseed, soft	8	70.0	39.0	1.00
Cottonseed, soft	9	57.9	44.0	3.00
Cottonseed, soft	10	60.5	38.0	2.75
Cottonseed, soft	11	65.0	41.0	2.40
Cottonseed, soft	12	73.0	39.6	3.50
Cottonseed, hard	1	5.8	61.0	3.00
Cottonseed, hard	3	...	61.0	5.00
Cottonseed, hard	5	37.5	59.0	2.80
Cottonseed, hard	6	8.0	63.0	0.75
Soy Bean, soft	1	70.3	39.4	4.50
Soy Bean, soft	2	72.0	42.0	1.00
Soy Bean, soft	5	83.0	46.0	5.00
Soy Bean, hard	1	4.4	62.5	0.55
Soy Bean, hard	3	10.0	65.5	1.25
Soy Bean, hard	4	29.5	...	3.50
Coconut	1	5.1	44.9	2.25
Coconut	2	4–12	33.0	3.25
Coconut	4[a]	1.5–3.5	38–39	15.00
Coconut	5[b]	3.0	49.0	0.60
Palm Kernel	1	0.5–1	47–8	trace
Palm Kernel	2	0.5–1	48.5–49.5	0.05
Peanut, soft		73.3	42.5	1.60
Sesame		57.7	45.6	0.70
Lard, soft	1	51.0	44.9	2.40
Lard, soft	2	58–61	47–48.5	8.00
Lard, hard	1	0.4	60.1	trace
Lard, hard	2	<2.0	61–2	1.65
Palm		2.0	59.0	2.40
Castor (3)		70.8	40.0	5.00
Castor (3)		16.6	82.0	8.50
Untreated Lard (3)		...	...	3.70
Untreated Petrolatum (3)		...	...	1.70

[a] With lecithin.
[b] With 6% cottonseed oil.

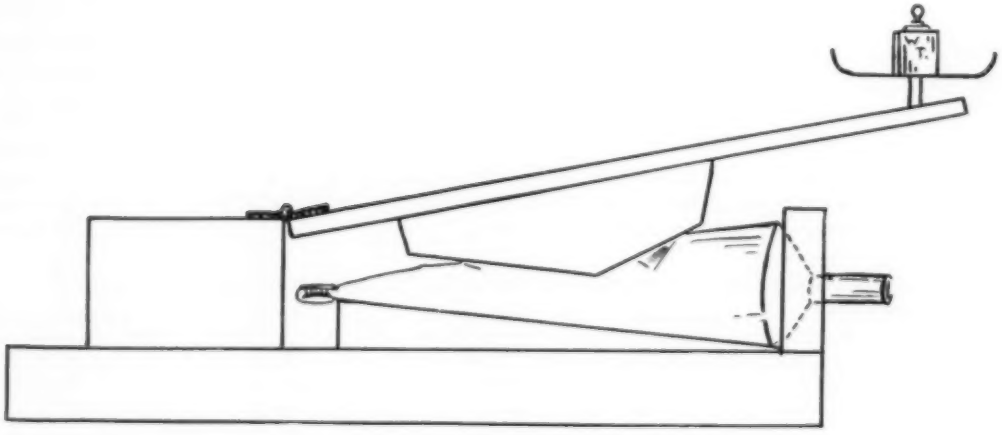

Fig. 1.—Compression-Consistency Apparatus.

SUMMARY

Commercial hydrogenated oils were studied to determine their suitability for ointments as far as stability to rancidity, consistency and water retention was concerned. None of the samples of hydrogenated vegetable oils developed more than a trace of rancidity when kept at body temperature in the dark in unstoppered glass tubes. Lard and partially hydrogenated lard, however, became distinctly rancid under the same conditions.

In sunlight all of the partially hydrogenated (I. V., more than 50) oils developed rancidity to some degree, but less rapid than lard. Hard hydrogenated oils (I. V., less than 10) did not develop rancidity as rapidly as partially hydrogenated oils. Hydrogenated oils of different manufacturers did not deteriorate exactly in proportion with the iodine value, but hydrogenated cottonseed oils of various iodine values prepared from the same batch of oil deteriorated in relationship to the iodine value, the lower iodine value oils being more stable.

Of all of the commercial partially hydrogenated oils, sesame oil was superior from the standpoint of rancidity. Samples of the same batch of oil hydrogenated to various degrees indicated that it was much more stable than hydrogenated cottonseed oil of the same iodine value.

Two pieces of apparatus are described for determining the relative consistency of fats. It was found that, in the main, the consistency varied with the softening point. The softening point of the same kind of oil from different manufacturers did not, as one would expect, vary with the iodine values, probably due to the differences in hydrogenating temperatures, differences in original oils, etc.

The data on water retention showed lack of uniformity with samples of different manufacturers. None of them retained as much water as hard hydrogenated castor oil with the exception of coconut oil containing lecithin which retained about five times as much as other coconut oils without lecithin. With the exception of partially hydrogenated lard, none of the partially hydrogenated oils retained as much water as partially hydrogenated castor oil.

Subsequent studies will be made to determine the relative value of various hydrogenated oils as bases for official ointments.

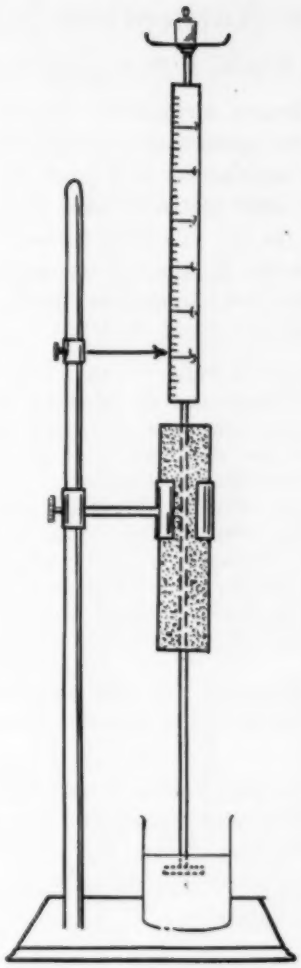

Fig. 2.—Penetration-Consistency Apparatus.

REFERENCES

(1) Fiero, JOUR. A. PH. A., 20, 254 (1931).

(2) *Pharmacopœia Helvetica*, 5th Edition, 1933.

(3) Fiero, JOUR. A. PH. A., 25, 862 (1936).

(4) Fiero and Lockie, *Ibid.*, 26, 402 (1937).

(5) *Official and Tentative Methods of the Am. Oil Chem. Soc.*, 34 (1932).

(6) Jamieson, G. S., *Vegetable Fats and Oils*, 1932.

(7) Fiero, *Am. J. Phar.*, 102, 146 (1930).

Liquor Antisepticus N. F. VI

*By C. O. Beebe, L. W. Busse and A. H. Uhl**

The present formula for Liquor Antisepticus in our opinion and the opinion of others is not as satisfactory as it might be. That it seems to have met with wide disapproval is attested by the following comments, which were received by the sub-committees of the N. F. Revision Committee shortly after the publication of the N. F. VI.

E. FULLERTON COOK: "I have had a number of complaints concerning the tendency of the new Liquor Antisepticus to become cloudy within a few hours, and also the unpleasant taste of the new solutions containing chlorthymol."

BERNARD FANTUS: "Having committed ourselves to the inclusion of chlorthymol and eucalyptol in Liquor Antisepticus would suggest that the maximum amount of these ingredients that remain in solution be specified in the formula."

S. L. HILTON: "If incompatible, delete chlorthymol."

H. R. WEMPLE: "No question about their incompatibility causing cloudiness to appear within a few hours."

After preparing several batches of Liquor Antisepticus and noting the incompatibilities referred to above, we carried out the following experiments in an attempt to improve the formula.

EXPERIMENTAL

These experiments consisted of a comparison of the antiseptic value of Liquor Antisepticus N. F. VI and modifications of it as directed by the general plan of disinfectant testing by the Hygienic Laboratory Method.

A test culture of *Bacillus coli* was used in place of *Bacillus typhosus*, Hopkins strain, as specified by the Hygienic Laboratory Method. Between periods of testing the culture was kept on nutrient agar slants, transferred at monthly intervals.

Phenol meeting the U. S. P. requirements was used in all experiments.

A 5% original solution was made by adding one part by weight of phenol, liquefied by warming the bottle, to nineteen parts of distilled water. A fresh solution was used for each day's testing.

Dilutions of phenol and disinfectant were made from the original liquid on the day of the test. The dilutions of phenol used were 1:80, 1:90, 1:100, 1:110, 1:120; 5 cc. of each was placed in a seeding tube, 1 x 3 inches with a flared top and a round bottom.

Dilutions of antiseptic solution used were, full strength, 1:2, 1:3, 1:4, 1:5; 5 cc. of each were placed in a seeding tube. All dilutions were made with sterile distilled water.

To test each tube, 0.1 cc. of culture was added seriatim, allowing 15 seconds for each addition. Five dilutions of antiseptic solution and 5 dilutions of phenol were used which required $2^1/_2$ minutes to carry out the procedure.

At the end of five minutes from the time the culture was added to the seeding tube, a mixture was transferred from the tube to a subculture tube and this procedure was carried out for each successive seeding tube at 15-second intervals. This procedure was repeated at $7^1/_2$, 10, $12^1/_2$ and 15 minutes from the time of the first addition of the culture to the seeding tube.

The tubes were properly labeled and placed in the incubator for 48 hours at 37° C., at the end of which time readings of growth or no growth were made and entered in a table as + or 0 signs, respectively.

The coefficient is the arithmetic mean of the sums of three ratios, expressed decimally. These ratios are the denominator of the highest dilution of the disinfectant in the subculture tube of which no growth occurs, divided by the corresponding figure for phenol, for the 5, 10 and 15-minute intervals, respectively.

Liquor Antisepticus and seven different modifications of it were prepared and tested. The modifications consisted solely of the deletion of one of the ingredients in an effort to determine what effect the various ingredients exerted upon the antiseptic properties of the solution. The following table is a summary of our results:

TABLE I.—EFFECT OF DELETING INGREDIENTS ON PHENOL COEFFICIENT

Expt. No.	Antiseptic Solution	Ingredient Deleted	Phenol Coefficient
1	N. F. VI	.....	0.023
2	N. F. VI	Boric acid	0.023
3	N. F. VI	Thymol	0.020
4	N. F. VI	Chlorthymol	0.012
5	N. F. VI	Menthol	0.036
6	N. F. VI	Eucalyptol	0.042
7	N. F. VI	Methyl salicylate	0.037
8	N. F. VI	Oil of thyme	0.027

From the above table we can draw the following conclusions:

1. Boric acid has no effect on the antiseptic value of the N. F. Antiseptic Solution.

2. The absence of either thymol or chlorthymol decreases the antiseptic value of the solution, the chlorthymol causing the greatest reduction.

3. Without menthol, eucalyptol, methyl salicylate or oil of thyme, N. F. Antiseptic Solution has a

* From the School of Pharmacy, University of Wisconsin. Presented before the Hospital Pharmacy Sub-Section, Aug. 25, 1939. The bacteriological work was carried out with the coöperation of Dr. P. V. Clark, Bacteriology Dept., College of Medicine, University of Wisconsin.

TABLE II.—EFFECTS OF DECREASED AMOUNTS OF THYMOL, CHLORTHYMOL AND MENTHOL

Solution	Boric Acid, Gm.	Thymol, Gm.	Chlorthymol, Gm.	Menthol, Gm.	Eucalyptol, Cc.	Methyl Salicylate, Cc.	Oil of Thyme, Cc.	Alcohol, Cc.	Phenol Coefficient
N. F. Bulletin Antiseptic Soln. Formula	25.00	0.5	0.5	0.5	0.2	1.2	0.3	250	0.049
Expt. 10	10.00	0.5	0.5	0.5	0.2	1.2	0.3	300	0.049
Expt. 11	25.00	1.0	...	0.5	0.2	1.2	0.3	250	0.024

TABLE III.—MAXIMUM QUANTITIES OF INGREDIENTS AND TEMPERATURES AT WHICH CLEAR SOLUTION RESULTS

Solution No.	Boric Acid, Gm.	Thymol, Gm.	Chlorthymol, Gm.	Menthol, Gm.	Eucalyptol, Cc.	Methyl Salicylate, Cc.	Oil of Thyme, Cc.	Alcohol, Cc.	Temp. Cloudy, ° C.	Phenol Coefficient
1	25.00	0.5	0.5	0.5	0.19	0.12	0.03	250.00	33	...
2	20.00	...	...	...	0.2	1.2	0.3	300.00	32	...
3	...	...	...	...	0.2	1.2	0.3	300.00	32	...
4	...	...	1.0	...	0.1	1.0	...	300.00	31	...
5	25.00	...	0.8	...	0.1	0.4	0.1	300.00	20	...
6	25.00	...	0.75	0.5	...	1.0	...	300.00	30	...
7	20.00	...	1.00	...	...	1.0	...	300.00	24	...
8	20.00	...	0.75	...	...	1.0	...	300.00	16	0.072
9	25.00	...	0.75	...	0.19	0.12	0.03	300.00	11	...
10	25.00	...	0.70	0.1	0.15	0.15	0.05	300.00	14	...
11	25.00	...	0.70	0.15	0.15	0.15	0.05	300.00	18	0.072
12	25.00	0.4	0.4	0.4	...	1.2	0.3	300.00	38	...

greater antiseptic value, the preparation without eucalyptol having the highest phenol coefficient.

After the effects of the deletion of the various ingredients of the formula on the phenol coefficient had been determined, our experiments were directed toward arriving at a formula which did not have the incompatibilities of the present formula, and which had as high or higher antiseptic value. Our efforts in solving the problem were directed toward using decreased amounts of the troublesome ingredients.

The effects of decreased amounts of thymol, chlorthymol and menthol are shown in Table II.

Decreasing the amounts of the chlorthymol and menthol from 1 Gm. to 0.5 Gm. and eucalyptol from 2 cc. to 0.2 cc. increased the antiseptic value of the solution. The solution, however, was cloudy and had to be clarified.

Reducing the boric acid to 10 Gm. and increasing the alcohol 50 cc. per L. gave a cloudy preparation which when clarified had no apparent effect on its antiseptic value.

Substituting 1 Gm. of thymol for 0.5 Gm. each of chlorthymol and thymol in Experiment 11 produced a cloudy solution which when clarified was of less antiseptic value than the former preparation.

Further results in the preparation of solutions, using decreased amounts of the various ingredients, are shown in Table III.

By comparing the amounts of the ingredients in the various preparations and noting the temperature at which they become cloudy, one arrives at the following conclusion:

1. By comparing solutions 2 and 3, it is seen that at room temperature a clear solution cannot be prepared using eucalyptol 0.2 cc., methyl salicylate 1.2 cc. and oil of thyme 0.3 cc. The temperature at which the product was clear was 34° C.

2. The reduction of eucalyptol to 0.1 cc., methyl salicylate to 0.4 cc., oil of thyme to 0.1 cc., using 0.8 Gm. of chlorthymol, 25 Gm. of boric acid and 300 cc. of alcohol, produced a clear solution at 22° C.

3. By comparing solutions number 9, 10, 11 and 12 it will be noted that the maximum amounts of ingredients giving a clear solution at 19° C. are those represented by solution 11.

GENERAL SUMMARY

1. N. F. Antiseptic Solution, before using purified talc to clarify the product, is a cloudy, milky appearing solution. The deletion of any one ingredient has little beneficial effect.

2. Boric acid exhibits no effect upon the antiseptic value (phenol coefficient) of N. F. Antiseptic Solution.

3. A higher antiseptic value, as determined by its phenol coefficient, will result upon the reduction of the amounts of volatile oils used in the preparation of N. F. Antiseptic Solution.

4. If a solution is to be prepared which will remain clear at ordinary temperatures without clarification, only certain amounts

of the ingredients in N. F. Antiseptic Solution can be used.

5. When decreased amounts of the ingredients in N. F. Antiseptic Solution are used, providing chlorthymol is not less than 0.5 Gm. per liter, the antiseptic value is equal to that of the present official solution.

The following formula with working directions is offered for criticism. This solution when submitted to the test for antiseptic value, described for N. F. Antiseptic Solution, showed *Staphylococcus aureus* to be killed in $^1/_2$ minute as shown by no growth on the subculture after incubation for 48 hours at 37.5° C.

Boric acid	25.00 Gm.
Chlorthymol	0.7 Gm.
Menthol	0.15 Gm.
Methyl salicylate	0.15 Gm.
Eucalyptol	0.15 Gm.
Oil of thyme	0.05 cc.
Alcohol	300.00 cc.
Distilled water *q. s.*	
To make	1000.00 cc.

Dissolve the boric acid in 650 cc. of warm distilled water and the other ingredients in the alcohol. Cool the boric acid solution, add it to the alcoholic solution with continual agitation; finally add sufficient distilled water to make 1000 cc. and mix well.

Assay of Sarsaparilla Preparations*

Preliminary Report

By B. Fantus, M.D., and H. A. Dyniewicz, Ph.C.

It is the consensus of modern medical opinion that the compound syrup of sarsaparilla is chiefly a flavoring vehicle (U. S. Dispensatory, page 1069, 21st Edition). This opinion was responsible for the deletion of the Fluidextract of Senna from the formula in the tenth revision as laxative effect might not be wanted in a mere flavoring agent. It may, indeed, be questioned whether the sarsaparilla might not be deleted from the formula, for the pleasant flavoring qualities of this syrup are not due to the sarsaparilla at all but are due to the volatile oils contained in the preparation. Indeed, what is by common consent called the sarsaparilla flavor is really a "sassafras bouquet," and the title "Compound Syrup of Sassafras" would be preferable unless it can be shown that sarsaparilla has some pharmacologic and possibly therapeutic action.

The question is whether the presence of the sarsaparilla is of any advantage in the compound syrup.

Prof. Gathercoal's prescription survey (1) shows the usage of the Compound Syrup of Sarsaparilla has been 60 per 10,000 prescriptions for the last 30 years. With this considerable usage in mind, in spite of the unfavorable opinion of the pharmacologists as to any medicinal value of sarsaparilla, we undertook this study in an endeavor to find upon what effect, if any, the preparations may be assayed.

According to a report by F. W. Apt (2) sarsaparilla is dependent upon saponin for its activity, and the saponin increases absorption of the medicament that may be carried in a preparation of sarsaparilla. The U. S. Dispensatory (21st Edition, page 963) reads: "The sarsaparilla of commerce is apt to be nearly, if not quite inert, either from age or from having been obtained from inferior species. The only criterion of good sarsaparilla to be relied on is its taste. As it leaves a decided acrid impression in the mouth it is considered effective; otherwise it is inert."

There are in the literature numerous references on the increased absorption of various drugs by means of saponins as will be seen by consulting the bibliography (3 to 10). Hence the sarsaparilla assay should be for saponin.

According to R. Kobert (11) the activity of the saponins is best evaluated by the hemolysis of red blood cells in physiologic salt solution in a given time. This was substantiated by C. Sormani and J. Rühle (12) and K. Hering (13). A test based upon hemolysis was developed by these investigators and modified by W. Brandt (14). A table of 80 drugs giving

* From the Laboratory of Pharmacology and Therapeutics, University of Illinois College of Medicine; assisted by a grant from the AMERICAN PHARMACEUTICAL ASSOCIATION.

the hemolytic index for each drug was reported by A. Kuhn and G. Schaefer (15).

The results obtained in our experiments were mostly for comparative purposes. They were all done on the same day with the same blood sample so that all observations were made under similar conditions giving relative values of the different saponins, and also fluidextracts and syrups of sarsaparilla.

A direct comparison of the fluidextract and syrup cannot be made since the rate of hemolysis is less in sucrose than in normal saline solution itself (16). Comparative results of various syrups, however, were secured since the quantity of sucrose was constant.

EXPERIMENTAL

Method.—Freshly drawn rabbit's blood was defibrinated by shaking with glass beads. The corpuscles from 2 cc. of this blood were washed several times with physiological salt solution and finally suspended in 100 cc. of physiologic salt solution.

To each test-tube containing 5 cc. of the defibrinated blood suspension an aqueous solution of the saponin (1 to 1000) was added in progressively increasing amounts: 0.1 cc., 0.2 cc., 0.3 cc., etc.; the mixture was made up to 10 cc. with physiologic salt solution. The saponins act directly on the cell according to E. Ponder (17) and belong to the simple hemolytic system. The criterion of complete lysis is the disappearance of all opacity or cloudiness in the tube. The tube containing the smallest amount of saponin hemolyzing completely in 5 minutes at room temperature, was taken as the reading and the "Hemolytic Index" calculated, *e. g.*,

0.1 cc. not complete in 5 minutes
0.2 cc. not complete in 5 minutes
0.3 cc. not complete in 5 minutes
0.4 cc. complete in 5 minutes
0.5 cc. complete in 5 minutes

The following results show the hemolytic index to be 0.0004 in 10 cc., or 1–25,000.

The hemolytic index is dependent upon the laking of blood due to the union of saponin with the cholesterin bodies. This we have found to vary for various specimens from 1:25,000 to 1:6250 (crude).

For the assay of fluidextracts, an aqueous dilution (2 to 100) of the fluidextract was used. The

TABLE I.—HEMOLYTIC INDEX (H. I.) OF VARIOUS SAPONINS

Saponin	Brand	Manufacturer's No.	In Dilution
No. 1	M-B	6080–3281	1–25,000
No. 2	M-B	6080–3281 (different container)	1–25,000
No. 3	M-B	6080–21848	1–14,300
No. 4	M-B	6081–32182	1– 9,100
No. 5	M-B	6081–32182 (different container)	1– 9,100
No. 6	M-B	6081–28264 (purified)	1–18,200
No. 7	F		1–14,300
No. 8	Crude		1– 6,250

This table shows that the same saponin, *i. e.*, having the same number as given it by a manufacturer gives the same assay even though taken from a different container.

TABLE II.—HEMOLYTIC INDEX OF VARIOUS FLUIDEXTRACTS

No. 1	(Reserved percolate, not subjected to heat)	1– 250
No. 2	(The combination of No. 1 and the pilular extract make the official fluidextract: only the extractive is subjected to heat)	1–1666
No. 3	(Jamaica sarsaparilla, wormy; fluidextract mucilagenous and murky)	1–1111
No. 4	(Honduras sarsaparilla)	1–1111
No. 5	(Mexican sarsaparilla)	1–1000
No. 6	(Commercial sample)	1– 666
No. 7	(Student's preparation—five years old)	1– 714
No. 8	(Student's preparation—one year old)	1– 333
No. 9	(Commercial sample)	1– 600
No. 10	(Commercial sample)	1– 294
No. 11	(Commercial sample)	1– 312
No. 12	(Laboratory, old sample)	1– 500

TABLE III.—HEMOLYTIC INDEX OF VARIOUS SYRUPS

No. 1	Syrup made from fluidextract No. 12 (months old)	1– 83
No. 2	Syrup made from fluidextract No. 12 (fresh)	1–100
No. 3	Syrup made from fluidextract No. 6	1–125
No. 4	Syrup from stockroom (old sample)	1– 62.5

hemolytic index of 12 different fluidextracts varied from 1:1666 to 1:300.

For the assay of syrups, an aqueous dilution (1 to 10) of the syrup was used. The hemolytic index of 4 different syrups varied from 1:62.5 to 1:125.

DISCUSSION

If saponin increases the absorption of drugs, then sarsaparilla may be more than a mere flavoring vehicle and should be assayed for its saponin. The hemolysis method seems to give satisfactory results. If accepted as an assay method for the official preparations, further experimentation will be necessary to standardize the assay. The results reported upon give relative values of the saponins, and also the fluidextracts and syrups of sarsaparilla. We have shown by means of the "hemolytic index" the variability of the saponins, fluidextracts and syrups of sarsaparilla upon the market to be so great that, if the medicinal effect of the preparations depended upon it, physicians might easily lose confidence in them. Further study of the possibility of using the hemolytic index for this purpose is indicated, and also a study as to whether the hemolytic index parallels the degree to which absorption of standard drugs are increased by the saponin.

BIBLIOGRAPHY

(1) Gathercoal, E. N., "The Prescription Ingredient Survey," AMERICAN PHARMACEUTICAL ASSOCIATION (1933).

(2) Apt, F. W., *Ber. deut. Pharm. Ges.*, 31, 155 (1921).

(3) Kofler, L., and Fischer, R., *Arch. Exptl. Path. Pharmakol.*, 141, 105–115 (1929). Cholesterol absorption favored.

(4) Hoekstra, R. A., *Ibid.*, 163, 46–65 (1931). Digitalis absorption favored.

(5) Lasch, F., *Biochem. Z.*, 169, 301–307 (1926). Calcium.

(6) Lasch, F., and Brügel, S., *Ibid.*, 172, 422–427 (1926). Dextrose.

(7) Lasch, F., and Brügel, S., *Arch. Exptl. Path. Pharmakol.*, 120, 144–155 (1927). Insulin.

(8) Köfler, L., *Deut. med. Wochschr.*, page 488 (Sept. 16, 1932). Acetylsalicylic acid, camphor, methylene blue, hypophysis.

(9) Annau, E., and Hergloz, J., *Arch. Exptl. Path. Pharmakol.*, 127, 93–100 (1927). Alkaloids, such as strychnine, curare, morphine, cocaine.

(10) Fantus, B., and Dyniewicz, H. A. and J. M., *J. Pharm. & Exp. Therap.*, 55, 222–233 (1935). Acetanilide.

(11) Kobert, R., *Pharm. Ztg.*, 57, 213–214 (1912).

(12) Sormani, C., and Rühle, J., *Ztschr. f. Unters. d. Nahr. u. Genussn.*, 23, 11 (1912).

(13) Hering, K., *Arch. Pharm.*, 268, 24–36 (1930).

(14) Brandt, W., *Pharm. Ztg.*, 69, 204–205 (1924).

(15) Kuhn, A., and Schaefer, G., *Ibid.*, 80, 257 (1925).

(16) Handovsky, Hans, *Arch. ges. Physiol.* (Pflüger's), 190, 173–176 (1921).

(17) Ponder, Eric, "The Mammalian Red Cell and the Properties of Hemolytic Systems," Vol. 6 (1934).

A Monograph for Ringer's Solution as a Solvent*

By Norman Pinschmidt and John C. Krantz, Jr.†

The first work of note with physiological saline solutions was done by Sidney Ringer and his associates between the years 1882–1895. They advocated the use of the chlorides of sodium, potassium and calcium and discussed the physiological results obtained by the addition of each of these salts to physiological solutions. Shafer (1) studied the solution as a solvent for various salts. Fantus and Dynievicz (2) suggested the use of Ringer's solution as a solvent for tannic acid in the treatment of burns. At the suggestion of the National Formulary Committee the following monograph was prepared for Ringer's solution, suitable for use as a solvent for hypotonic solutions to be used parentally or when chlorides of sodium, potassium and calcium in the tissues have been diminished.

LIQUOR PHYSIOLOGICALIS SALINIS

Solution of Physiological Salts

Ringer's Solution

Solution of Physiological Salts contains in 100 cc. not less than 0.84 or more than 0.88 Gm. of NaCl, not less than 0.025 and not more than 0.035 Gm. of KCl and not less than 0.030 or more than 0.036 Gm. of $CaCl_2.2H_2O$.

* The expense of this investigation was defrayed in part by a grant from the National Formulary Committee.

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Sodium chloride	8.6 Gm.
Potassium chloride	0.30 Gm.
Calcium chloride (dihydrate)	0.33 Gm.
Recently boiled distilled water, sufficient to make	1000 cc.

Dissolve the three salts in a convenient quantity of recently boiled, previously cooled distilled water, to make up to 1000 cc. and filter. The solution may be sterilized by placing in an autoclave at 15 lbs. pressure for 15 minutes.

Description and Physical Properties.—Solution of Physiological Salts is a clear, colorless solution possessing a mild saline taste. The specific gravity of the solution at 25° C. is about 1.005. The hydrogen-ion concentration of the solution is about p_H 6.5. The solution gives a depression of freezing point not less than −0.54° C. and not more than −0.58° C.

Heavy Metals.—Ten cc. of Solution of Physiological Salts meets the requirements for the test for heavy metals.

Arsenic.—Twenty cc. of Solution of Physiological Salts meets with the requirements of the test for arsenic.

Tests for Sterility.—For bacterial contamination and for yeasts and molds proceed according to the methods given under Ampuls of Dextrose.

Assay for Calcium Chloride.—Evaporate 100 cc. of Solution of Physiological Salts accurately measured to 20 cc., heat to boiling and make alkaline with ammonia T.S., add ammonium oxalate T.S. dropwise until all the calcium has been precipitated. Heat on a water-bath for an hour, filter through hardened filter paper and wash thoroughly with warm distilled water. Puncture the filter and wash the precipitate into a beaker by means of a stream of hot distilled water followed by 30 cc. of dilute sulfuric acid. Heat to 60° C. and titrate with one-hundredth-normal potassium permanganate. Each cc. of one-hundredth-normal potassium permanganate is equivalent to 0.00074 Gm. $CaCl_2 \cdot 2H_2O$.

Assay for Potassium Chloride.—To 1.5 cc. of alcohol in a 15-cc. centrifuge tube add 5 cc. of Solution of Physiological Salts accurately measured, and mix thoroughly. Add dropwise, with continuous shaking, 2 cc. of potassium precipitating reagent. Allow to stand for one hour at room temperature. Centrifuge for about 10 minutes at about 2000 r. p. m. or until the precipitate is firmly packed in the bottom of the tube. Decant the supernatant liquid and allow the precipitate to drain for about five minutes. Wash the precipitate carefully with 5 cc. of 70 per cent alcohol, breaking up the bulk of the precipitate by forcing the wash solution in a fine stream from the pipette. Centrifuge for five minutes and drain as before. Dry the precipitate for one hour at 80 to 85° C. to remove all of the alcohol.

Add 5 cc. of two-hundredth-normal ceric sulfate and 1 cc. of sulfuric acid which has been previously diluted with an equal volume of distilled water. Heat on a water-bath until all the precipitate has disappeared. Cool to room temperature and titrate with ferrous ammonium sulfate solution using one drop of *o*-phenanthroline ferrous complex T.S. as indicator. Each cc. of two-hundredth-normal ceric sulfate used to oxidize the precipitate multiplied by 0.249 equals the weight of KCl in milligrams.

Assay for Total Chlorides.—Transfer 10 cc. of Physiological Salts Solution accurately measured to a 400-cc. beaker, add 50 cc. of distilled water and 4 cc. of diluted nitric acid; dilute to 200 cc. with distilled water, add 15 cc. of silver nitrate T.S., heat to boiling and allow to stand protected from direct light until the precipitate is granular. Filter onto a tared Gooch crucible previously heated to 150° C. Dry the precipitate to a constant weight at 150° and weigh. The weight of silver chloride obtained multiplied by 0.2474 equals the weight of total chloride. The chloride (Cl^-) calculated from the silver chloride is not less than 0.0520 Gm. or more than 0.0580.

Storage.—The solution should be kept in hard, glass-stoppered bottles.

TABLE I.—REAGENTS, INDICATORS AND STANDARD SOLUTIONS REQUIRED BUT NOT IN NATIONAL FORMULARY

I. Reagent:

Potassium Precipitating Reagent:

Dissolve 11.6 Gm. sodium cobaltinitrite, 4.75 Gm. sodium acetate and 4.5 cc. glacial acetic acid in 30 cc. of distilled water, allow to stand 48 hours and centrifuge at 2000 r. p. m. before using. Keep in a cold, dark place.

II. Indicators:

o-Phenanthroline Ferrous Complex:

See U. S. P. XI, Second Supplement.

III. Standard Solutions:

Ceric Sulfate, Two-hundredths-normal:

$Ce(SO_4)_2 = 333.25$

6.646 Gm. of $Ce(SO_4)_2$ in 1000 cc.

One cc. of 0.02*N* ceric sulfate is the equivalent of ceric sulfate, $Ce(SO_4)_2$..... .....0.0006646 Gram.

Preparation: See U. S. P. XI, Second Supplement.

EXPERIMENTAL

Table I shows the results obtained from assays on five different Solutions of Physiological Salts at optimum conditions.

In determining a suitable formula for the solution comparative studies were made of the large number of formulas available. It was decided after examination of the magnesium present as impurity in the salts of sodium and potassium to omit the magnesium from the formula.

TABLE II.—ANALYTICAL DATA ON SOLUTION OF PHYSIOLOGICAL SALTS

Bottle No.	Calcium Chloride Assay, Gm. (10 Cc. Aliquot)	Potassium Chloride Assay, Mg. (5 Cc. Aliquot)	Total Chlorides Assay, Gm. (10 Cc. Aliquot)	Depression Freezing Point, ° C.	p_H
1	0.0343	1.54	0.0532	0.56	6.5
2	0.0341	1.53	0.0533	0.57	6.6
3	0.0354	1.54	0.0538	0.56	6.0
4	0.0337	1.53	0.0532	0.56	6.4
5	0.0343	1.56	0.0529	0.56	6.4

The addition of sodium bicarbonate was not deemed advisable because of its alkalinity. Buffer salts were considered but in all probability unnecessary as the p_H of the solution is quite constant.

The sodium chloride suggested is of the U. S. P. grade. Comparative studies of several commonly used brands of the C.P. salt show that the U. S. P. standard was in most cases slightly alkaline whereas the C.P. standard was slightly acid. This sodium chloride alkalinity tends to combat the acidity found in all commercial samples of calcium chloride.

Concerning the calcium chloride used these observations revealed that all hydrates of calcium chloride ($CaCl_2$; $CaCl_2.2H_2O$; $CaCl_2.6H_2O$) seemed to impart a slight acidity to the solution. The hydrated gave a lesser degree of acidity than the anhydrous salt. Of the two hydrates the dihydrate is the better.

The solution has been made isotonic with blood serum and the isotonicity checked cryoscopically and through its speed of hemolysis of red blood cells.

In the assay for the potassium salt seven different procedures were tried with consistently low results and a high percentage error. The method suggested by Brown, Robinson and Browning (3), however, if followed carefully will give consistent and accurate results.

In the consideration of the use of Ringer's Solution as a solvent for the various ampul solutions of the National Formulary, there are only two ampul solutions in the group that are hypotonic, namely, emetine hydrochloride and mercuric succinimide. The latter, however, is decomposed in the presence of sodium chloride giving ionizable mercury and could not, therefore, be made isotonic by the addition of Solution of Physiological Salts.

The emetine hydrochloride, however, may be adjusted to isotonicity by the addition of 28.2 cc. of Ringer's Solution per 100 cc. of solution. This isotonic emetine hydrochloride in Ringer's Solution was prepared and checked cryoscopically.

CONCLUSION

A formula and monograph for a Physiological Salts Solution as a solvent for parenteral use have been proposed for the National Formulary.

BIBLIOGRAPHY

(1) Shafer, G. D., *Am. J. Physiol.*, 87, 150-171 (1928–1929).

(2) Fantus, B., and Dyniewicz, H. A., *J. Am. Med. Assoc.*, 109, 200 (1937).

(3) Brown, D. S., Robinson, R. R., and Browning, G. M., *Ind. Eng. Chem., Anal. Ed.*, 10, 652 (1938).

A Review of Tentative Changes in the Ampul Monographs of the National Formulary

By R. K. Snyder and E. N. Gathercoal*

INTRODUCTION

The Special N. F. Committee on Ampuls and Tablets[1] had their first meeting in January 1936 and laid the foundation for the work that has been done since that time. The ampul monographs, including the general monograph, were to be studied in detail and desirable changes were to be recommended to the N. F. Committee. This work has been completed and is set forth in a tentative form in the Bulletin of the National Formulary Committee (1).

The laboratory work involved was begun in the N. F. Laboratory in Chicago and completed in the A. PH. A. Laboratory in Washington. Collaboration on suggested assay methods was conducted in the Laboratories which the committee members represented. The results were discussed and decisions reached in the six semiannual meetings of the Committee (2).

GENERAL DISCUSSION

In a broad sense the work involved standards, methods of preparation, tests for identity and purity of the drugs used, methods of assay, sterilization procedures, and methods for testing the suitability of the glass containers.

To serve as a starting point a supply of the various official ampuls was obtained from each of five commercial houses.

The **tests of identity and purity** as listed in the monograph of each drug in the U. S.

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[1] The members of this committee are: E. N. Gathercoal, Russel A. Cain, F. E. Bibbins, F. O. Taylor, J. B. Fullerton, E. B. Carter and R. K. Snyder.

P. XI or N. F. VI were applied to these ampul solutions. It was found that practically all of the identity and purity tests could be performed on the chemicals in the solutions and yielded satisfactory results. There were a few tests, such as "moisture content," which obviously could not be used on a solution of the chemical or because another chemical was present which would vitiate the test.

The **official assays** were carried out on each commercial lot of each ampul solution and the results were carefully studied. It was apparent that some of these methods required modification or replacement to increase accuracy of the assay. The literature was reviewed and other assay methods were tried out. From these results certain assays were selected and collaborative work was done by the committee members. Of the revised assays presented later in the paper, only two can be considered as being new, the others are modifications of assays presented in the literature. It should be noted that the assays of similar ampul solutions were made identical, such as the calcium salt assays and the chloride and iodide assays.

The **directions for preparation of the ampul solutions** were also investigated to determine if they were satisfactory. Particular attention was paid to the oil suspensions in regard to smoothness, uniformity, particle size and any tendency for the particles to clump or aggregate.

The **testing of small and large glass containers** of ampul solutions has been discussed in previous papers (3) by the authors. After collaboration with chemists in the laboratories of glass manufacturers, as well as of ampul manufacturers, a method has been selected for ampuls up to 100-cc. capacity and a second method for containers of 100 cc. to 1000-cc. capacity. Further collaborative work is in progress at the present time to determine the effect on the surface durability of treated soft glass containers by aging common parenteral solutions in them.

It is of interest to note that no deletions of ampul monographs have been recommended, but that three new monographs have been suggested and prepared. These are Ampuls of Bismuth and Potassium Tartrate in Oil, Ampuls of Sodium Salicylate and Iodide, and Ampuls of Sodium Salicylate and Iodide with Colchicine.

Tests for Ampul Glass.—The glass in ampuls of a capacity of 100 cc. or less shall meet the following test for solubility and reaction as a minimum requirement of quality:

Take a sufficient number of ampuls, not less than six, cleanse them thoroughly, rinse in distilled water and dry. Coarsely crush the dry ampuls, mix the crushed glass well, and reduce a sufficient quantity of it to a powder in a suitable steel mortar or mill. Sift the powdered glass and reserve the portion that will pass through a No. 40 standard mesh sieve, but will not pass through a No. 60 standard mesh sieve. Free this portion of glass from particles of steel with a magnet, wash it with alcohol to remove the finer dust particles and dry it in an oven. Place 10 Gm. of the dry glass, accurately weighed, in a resistance glass flask of about 125-cc. capacity, add 40 cc. of ampul water, loosely cap the flask with a glass or metallic cover, and autoclave for 30 minutes at 15 lbs. pressure (121° C.). Cool the autoclave to atmospheric pressure within 30 minutes, then remove the flask from the autoclave at once, cool rapidly to room temperature and titrate the contents of the flask with fiftieth-normal sulfuric acid, using phenol red p_H indicator. Also run a blank using the same kind of flask and cover and 40 cc. of ampul water from the same lot. Deduct the amount of fiftieth-normal sulfuric acid consumed in the blank from that consumed in the solution of the glass: the difference is not more than 0.75 cc.

Glass containers for solutions or suspensions intended for parenteral use and of a capacity greater than 100 cc. shall meet the following test:

Cleanse not less than six of the containers thoroughly with hot water and rinse them with distilled water. Nearly fill them with ampul water, invert Pyrex beakers over the openings of the containers, and autoclave them for 30 minutes at 15 lbs. pressure (121° C.). Cool the autoclave to about 100° C. within 30 minutes and then remove the containers from the autoclave at once. Transfer an accurately measured suitable quantity, but not less than 100 cc. from each container, to a pyrex beaker, acidify the solution with not more than 1 cc. of sulfuric acid, evaporate the solution to a volume of 30 to 40 cc. and carefully transfer the contents to a previously ignited, accurately weighed, platinum dish. Evaporate to dryness, ignite at a dull red heat until constant weight is obtained and express the total solids as parts per million of sulfates. In like manner determine the total solids as parts per million of sulfates, in the ampul water from the same lot as used in the test. Deduct the latter figure from the former: the difference is not more than 3.5 parts per million.

Assay of Ampuls of Bismuth and Potassium Tar-

trate in Oil.—Transfer an accurately measured volume of the uniform suspension containing about 0.2 Gm. of bismuth and potassium tartrate, to a crucible and add sufficient shredded filter paper to absorb the oil. Touch a flame to the oil-soaked paper, and when the mass is carbonized, ignite it. Cool, add nitric acid, cover the crucible with a watch glass and heat on a water-bath until the residue dissolves. Rinse the crucible into a filter and carefully wash the filter. Add stronger ammonia water to the filtrate until a slight but permanent precipitate is produced, then add 2 cc. of nitric acid and increase the volume to about 100 cc. Heat the solution to boiling and add 50 cc. of tenth-molar dibasic ammonium phosphate (also heated to boiling) slowly during the course of three minutes. Digest the mixture at 80° C. for one hour. Allow to settle and decant through an ignited, tared Gooch crucible, washing the precipitate three times with 50-cc. portions of hot water. Transfer the precipitate to the crucible with cold water, wash with cold water, dry and ignite the crucible to dull redness for a half-hour and weigh the bismuth phosphate, $BiPO_4$.

Assay of Ampuls of Bismuth Subsalicylate.—Transfer an accurately measured volume of the uniform suspension containing about 1 Gm. of bismuth subsalicylate, to a tared crucible. Add sufficient shredded ashless filter paper to absorb the suspension. Touch a flame to the oil-soaked paper, and when the mass is carbonized, ignite it. Cool, add 3 cc. of nitric acid, drop by drop, and warm until the residue is dissolved. Evaporate the solution to dryness, ignite the residue at red heat and weigh as Bi_2O_3.

Assay of Ampuls of Calcium Chloride.—Transfer an accurately measured volume of the ampul solution, containing about 0.25 Gm. of calcium chloride, to a beaker. Add 2 cc. of hydrochloric acid and 100 cc. of distilled water, heat to boiling, make alkaline with ammonia T.S., and add, with stirring, an excess of hot ammonium oxalate T.S. Heat the mixture on a water-bath for one hour, filter, and wash the precipitate on the filter with warm distilled water until the washings give no precipitate with calcium chloride T.S. Transfer the precipitate and filter to a beaker, add 100 cc. of distilled water and 10 cc. of sulfuric acid. Heat the solution to 70° C. and titrate with tenth-normal potassium permanganate.

Each cc. of tenth-normal potassium permanganate is equivalent to 0.005550 Gm. of $CaCl_2$.

Assay of Ampuls of Calcium Gluconate.—Transfer an accurately measured volume of the ampul solution, containing about 0.5 Gm. of calcium gluconate, to a beaker and proceed with the assay as directed under Ampuls of Calcium Chloride.

Assay of Ampuls of Ephedrine Sulfate.—Transfer an accurately measured volume of the ampul solution, containing about 0.25 Gm. of ephedrine sulfate, to a separatory funnel or a continuous extraction apparatus, add sufficient distilled water to make about 10 cc. and then 3 cc. of half-normal sodium hydroxide and extract the alkaloid completely in the continuous extraction apparatus, or by shaking with six successive portions of ether using 20 cc., 15 cc., 10 cc., 10 cc., 10 cc. and 10 cc., respectively. Collect the ether extractions in a separatory funnel, wash the combined extractions with 5 cc. of distilled water and transfer the washings to a second separatory funnel. Extract the water washings with 10 cc. of ether and add this ether extraction to the combined ether extractions in the first separatory funnel. Discard the residual water. Extract combined ethereal solutions with 15 cc. of tenth-normal sulfuric acid, followed by 10 cc. and 5 cc. portions of distilled water. Collect these acid and water extractions in a beaker and gently evaporate on a water-bath until the odor of ether is no longer perceptible. Cool and titrate the excess acid with fiftieth-normal sodium hydroxide using methyl red T.S. as the indicator.

Each cc. of tenth-normal sulfuric acid is equivalent to 0.01651 Gm. of $C_{18}H_{15}ON$.

Assay of Ampuls of Green Iron and Ammonium Citrates.—Transfer an accurately measured volume of the ampul solution containing about 1 Gm. of Green Iron and Ammonium Citrates, to a glass-stoppered separatory funnel, add ammonia water to render alkaline and extract the alkaloid by shaking with three portions of chloroform. Wash the combined chloroformic extracts once with distilled water, add the washing to the alkaline liquid in a glass-stoppered flask, neutralize it with hydrochloric acid and add an excess of 5 cc. of acid; dilute with distilled water, if necessary, to make 25 cc.; then add 4 Gm. of potassium iodide and stopper the flask securely. Allow the mixture to stand for from twenty to thirty minutes; then add 50 cc. of distilled water and titrate the liberated iodine with tenth-normal sodium thiosulfate, using starch T.S. as the indicator.

Each cc. of tenth-normal sodium thiosulfate is equivalent to 0.005584 Gm. of Fe.

Assay of Ampuls of Mercuric Salicylate.—Transfer an accurately measured volume of the suspension, containing about 0.3 Gm. of mercuric salicylate, to a prepared Gooch filter and wash with purified petroleum benzin to remove all of the oil. Place the Gooch filter and residue into a suitable flask and add 15 cc. of sulfuric acid and 15 cc. of nitric acid. Insert a small funnel in the neck of the flask and heat upon a sand-bath until the mixture is decolorized. Cool the solution, dilute it with 150 cc. of distilled water, add 2 cc. of ferric ammonium sulfate T.S. and titrate with tenth-normal ammonium thiocyanate.

Each cc. of tenth-normal ammonium thiocyanate is equivalent to 0.01003 Gm. of Hg.

Assay of Ampuls of Sodium Chloride.—Transfer an accurately measured volume of the ampul solution, suitably diluted with distilled water and containing about 0.15 Gm. of sodium chloride, to a casserole, add 200 cc. of distilled water and 10 drops

of dichlorofluorescein indicator. Titrate the mixture with tenth-normal silver nitrate solution until the silver chloride flocculates and the mixture turns to a faint pink.

Assay of Ampuls of Sodium Iodide.—Transfer an accurately measured volume of the ampul solution, containing about 0.25 Gm. of sodium iodide, to a casserole, add 200 cc. of the distilled water and 10 drops of dichlorofluorescein indicator. Titrate the mixture with tenth-normal silver nitrate solution until the silver iodide flocculates and the mixture turns a faint pink.

Each cc. of tenth-normal silver nitrate is equivalent to 0.01499 Gm. of NaI.

Assay of Ampuls of Sodium Salicylate.—Transfer an accurately measured volume of the ampul solution, containing about 0.25 Gm. of sodium salicylate, to a 100-cc. glass-stoppered cylinder and add 75 cc. of ether and 5 drops of bromphenol blue T.S. as the indicator. Titrate the mixture with tenth-normal hydrochloric acid, mixing intimately the aqueous and ethereal layers by vigorous shaking, until a permanent pale green color is produced in the aqueous layer.

Each cc. of tenth-normal hydrochloric acid is equivalent to 0.01601 Gm. of $C_6H_4.OH.COONa$.

BIBLIOGRAPHY

(1) Bulletin of the National Formulary Committee, Vol. VII, No. 5 (February 1939). Copies of this number of the Bulletin may be obtained from Secretary E. F. Kelly of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., Washington, D. C.

(2) Bulletin of N. F. Sub-committee on Ampuls and Tablets, pp. 1–99, 109–202. N. F. Bull., Vol. V, pp. 2215, 2261, 2270–2275, 2298, 2329, 2340–2346, 2412–2413, 2435; Vol. VI, pp. 2500, 2548, 2580, 2584–2589, 2590–2598, 2648–2661, 2752–2777, 2958; Vol. VII, pp. 8, 9, 10, 11, 22, 168–189.

(3) JOUR. A. PH. A., 26, 321–328 (April 1937); 28, 7–11 (Jan. 1939).

Determination of Acetone

*By Melvin W. Green**

INTRODUCTION

Three methods are in general use for the determination of acetone. The official method depends upon the conversion of the acetone to iodoform with an excess of iodine and the subsequent determination of the iodine with thiosulfate. Another method makes use of the qualitative test for acetone in which the acetone is precipitated as a yellow mercury complex. The mercury in this complex is then determined mercurimetrically or gravimetrically. The third method takes advantage of the carbonyl group which reacts with hydroxylamine hydrochloride to form acetoxime, liberating hydrochloric acid which is titrated with alkali.

Since there has been some criticism of the present Pharmacopœial method, a comparison of the three methods was undertaken by this laboratory.

* Cincinnati College of Pharmacy.

EXPERIMENTAL

Purification of Sample.—A commercial acetone, alleged to be of U. S. P. grade, was refluxed for two hours with calcium oxide and then fractionally distilled, collecting only that fraction which boiled at 56.3 to 56.4° C. (uncor.).

Mercurimetric Method.—In 1926, Jonescu-Matin (1) recommended the determination of acetone, as well as certain alkaloids, by forming the mercury complex and then determining the mercury contained therein.

The reagent was prepared by mixing 50 Gm. of mercuric oxide with 500 Gm. of concentrated sulfuric acid and then diluting to one liter with water. In conducting the test, 1 to 10 cc. of a 5% aqueous acetone were refluxed for twenty minutes in the presence of 10 cc. of 50% sulfuric acid, 10 cc. of the mercury reagent and enough water to make a total of 100 cc. The material in the flask was then cooled, filtered, washed with 100 cc. of water in divided portions, the residue washed into a flask by a stream of water from a wash bottle and digested with 25 cc. of a mixture of two parts sulfuric acid to one part of nitric acid until solution was effected. The mercury was then determined by the familiar thiocyanate method, using ferric alum as the indicator. Jonescu-Matin claims the complex to have the formula $Hg_2SO_4{:}3HgO.4(CH_3)_2CO$. This would correspond to an acetone content of nearly 17%. However, the author experienced a higher and variable result, so variable that the method was abandoned as too unpredictable and too time-consuming for practicality.

Oxime Method.—In the oxime method, advantage is taken of the reactivity of the carbonyl group with hydroxylamine hydrochloride according to the following equation:

$$(CH_3)_2CO + NH_2OH.HCl = (CH_3)_2C = NOH + HCl \quad (1)$$

The HCl thus liberated may be titrated with 0.1*N* NaOH using methyl orange as an indicator, although various other single and a few mixed indicators have been advocated. However, according to Morasco (2) the reaction is only 94.4% complete.

The reaction was conducted as follows: Approximately 2 cc. of the purified acetone were added by

pipette to a weighed 200-cc. volumetric flask containing about 50 cc. of distilled water. The flask was shaken and then made up to the mark with water. In each of two beakers, 400 cc. of a 0.2% hydroxylamine hydrochloride solution were placed and the contents of one beaker was neutralized to the indicator and to the other beaker were added 25 cc. of the aqueous acetone solution previously prepared. After standing fifteen minutes, the acid liberated by the reaction was titrated to match the control fluid. One cc. of 0.1*N* NaOH = 0.00614 Gm. of acetone.

Using this technique, values from 100.5% to 101.8% of theory were obtained. It is worthy of note that the Morasco factor is based upon the assumption that the iodoform reaction is exactly correct (*i. e.*, 1 cc. of 0.1*N* I_2 = 0.009675 Gm. of acetone). However, Haughton (3) believes the reaction to go to 97.1% completion rather than 94.4% and that, therefore, the factor should be 0.00598. The work in this laboratory seems to substantiate a need for a lower factor. However, here it was found that the factor varied with the sample of hydroxylamine hydrochloride and with the concentration of acetone employed.

Any attempt to change the time, temperature or concentration factors failed to yield a constant result. Consequently, it was concluded that such a method would be of little value officially unless some method of control could be found that would yield at least a constantly dependable and reproducible factor.

Messinger's Iodoform Method.—The technique used was that described in the U. S. P. XI. The results obtained by following directions closely are presented in Table I.

TABLE I.—IODIMETRIC DETERMINATION OF ACETONE

No.	Acetone Added, Gm.	Acetone Recovered, Gm.	Per Cent of Theory
1	0.01968	0.01973	100.25
	0.01968	0.01972	100.20
2	0.01982	0.01986	100.18
	0.01982	0.01987	100.25
3	0.01990	0.02001	100.55
	0.01990	0.01996	100.31

It was thought that perhaps the amount of alkali was a factor. To determine the effect of the alkali concentration, the assay was conducted as directed in the U. S. P., excepting that the alkali (and acid) concentrations were varied from 0.1*N* to 2.5*N*. Using 0.1*N* alkali, only about 40% of theory was obtained. The conditions under which exactly six atoms of iodine react with one molecule of acetone, were considered to give 100% of theory. Constancy of values arises at about 100.8% of theory in alkali concentrations from 0.85*N* to 2.5*N* NaOH. The alkali value corresponding to exactly 100% varied from about 0.6*N* to 0.85*N* and was too variable to be dependable. Consequently, it would seem that 1*N* alkali is necessary to be assured of constancy.

Changes in the time which the iodine was in contact with the alkaline acetone and the speed of the addition of the iodine produced no material deviation from the foregoing results.

The results of this reaction would, of course, be high in the presence of alcohol. Rakshit (4) has modified the U. S. P. method by replacing the sodium hydroxide with lime water, claiming that the presence of alcohol does not then interfere. However, it was the experience of this laboratory that lime water gave an impossibly low result.

For estimation of small quantities of alcohol in the presence of acetone, Craven (5) observed that 1 cc. of pure acetone with 3 cc. of a reagent containing 0.5 Gm. of potassium dichromate in 100 cc. of pure colorless nitric acid (sp. gr. 1.310) developed a green color only after three hours whereas with 0.5% alcohol or formaldehyde, a blue color developed almost immediately. The author applied the reaction to both commercial acetone U. S. P. and the purified acetone. No green color developed with the acetone as long as the nitric acid was free from colored nitrogen oxide fumes. It was found that the threshold concentration of alcohol, 4:1000, developed a blue color within five minutes. Since the commercial acetone never developed the color within the time limit, it was concluded that the alcohol concentration was never over 4:1000 and that, therefore, any high results could not be due to the high alcohol content.

Haughton (3) has suggested that in addition to the main reaction a side reaction takes place as follows:

$$CH_3COCH_3 + 5NaOH + 5I_2 = 2CHI_3 + HCOONa + 4NaI + 4H_2O \quad (2)$$

This side reaction consumes ten atoms of iodine instead of the six of the normal reaction and hence would possibly account for the high results.

If this side reaction occurs, one should be able to detect formic acid in the reaction mixture. To test for formate, 2 cc. of acetone were placed in excess alkali and an excess of 0.1 *N* iodine added. After about thirty minutes, the iodoform was filtered off. After acidifying the filtrate with sulfuric acid, mercury was added to combine with any free iodine and the mixture filtered and steam-distilled. The distillate was made alkaline to litmus with caustic, a slight excess added and evaporated down to about 200 cc. This liquid was refluxed for one hour in the presence of 5% mercuric chloride after first neutralizing with hydrochloric acid. By this method, 1.55 Gm. of acetone yielded 0.068 Gm. of mercurous chloride. A blank made by distilling acetic acid, in the approximate concentration obtained from the normal iodination of acetone, yielded only the slightest trace of turbidity equivalent to less than 0.1 mg. of calomel.

From these results, it would seem that the side reaction probably does occur to a limited extent. It is interesting to note that Cassar (6) had a similar experience in determining methyl ethyl ketone in the presence of secondary butanol. In this case the main reaction is:

$$CH_3COCH_2CH_3 + 3I_2 + 3NaOH = CHI_3 + CH_3CH_2COOH + 3NaI + 2H_2O \quad (3)$$

and the secondary reaction is:

$$CH_3COCH_2CH_3 + 5I_2 + 4NaOH = 2CHI_3 + CH_3COOH + 4NaI + 3H_2O \quad (4)$$

Cassar found that the iodoform method yielded about 110.6% of theory and he believes that the high results may be attributed to the secondary reaction and its subsequent consumption of more iodine.

SUMMARY

1. There are three general methods for assaying acetone: the mercury complex method, the oxime method and the iodoform method.

2. The mercury method gives the lowest results, the oxime method high results and both give variable results.

3. The iodoform method gave the most uniform results, but the results are high. It is believed that these high results are due to a secondary reaction involving formate and consuming ten rather than six atoms of iodine.

BIBLIOGRAPHY

(1) Jonescu-Matin, A. L., *J. Pharm. Chem.*, 4, 533 (1926).

(2) Morasco, M., *Ind. Eng. Chem.*, 18, 701 (1926).

(3) Haughton, C. O., *Ind. Eng. Chem.*, Anal. Ed., 9, 167 (1937).

(4) Rakshit, J., *Analyst*, 41, 245 (1916).

(5) Craven, E. C., *J. Soc. Chem. Ind.*, 52, 239T (1933).

(6) Cassar, H. A., *Ind. Eng. Chem.*, 19, 1061 (1927).

Decomposition Rate of Ethyl Nitrite in Brown Mixture, U. S. P.

By Edward Greenfield and H. Walter Kuhl**

Several samples of Brown Mixture, U. S. P. XI, known to be approximately six months old, were tested for their ethyl nitrite content. Analysis showed none of these samples to contain any ethyl nitrite. In view of this, experiments were made to determine the rate of decomposition of this ingredient in the mixture.

EXPERIMENTAL

A modified Peter Griess (1) method for the determination of nitrous acid was employed in these experiments. This colorimetric method is extremely sensitive, depending upon the formation of intensively colored azo dyes (2) and detecting as little as 0.001 mg. HONO in a liter. It was assumed that the ethyl nitrite was hydrolyzed to nitrous acid by the action of the acetic acid in the reagent. A standard solution of sodium nitrite was used for quantitative colorimetric comparison.

Procedure.—A well-shaken sample of Brown Mixture was diluted to 4000 volumes to yield a theoretical concentration of 0.3 mg. C_2H_5ONO per liter in a practically colorless solution. Fifty cc. of this latter solution, theoretically containing 0.015 mg. C_2H_5ONO, was used for the actual test. After negative results were obtained with the original samples of Brown Mixture, the theoretical amount of Spirit of Nitre, as per U. S. P., was added to a composite of these samples. At the same time a fresh batch of Brown Mixture was prepared by the official U. S. P. method. These two samples were individually assayed each day, after preparation, up to a month. The composite results follow:

RATE OF DECOMPOSITION OF ETHYL NITRITE IN BROWN MIXTURE

Time after Manufacture of Mixture	Ethyl Nitrite Remaining, in Per Cent
0	100.0
4 Hours	66.5
28 Hours	39.5
2 Days	33.3
5 Days	13.3
6 Days	6.7
1 Month	Negligible

From the above results, it is evident that the ethyl nitrite begins to decompose as soon as it is added to the preparation. It is doubtful whether it has any therapeutic (3), (4) or other value in the preparation unless it is used immediately after manufacture. It has been shown (5) that on deterioration ethyl nitrite develops no new kind of pharmacologic activity.

CONCLUSION

The decomposition rate of ethyl nitrite in Brown Mixture U. S. P. is so rapid and extensive as to render it valueless in the latter preparation.

REFERENCES

(1) Treadwell and Hall, "Analytical Chemistry," Vol. II, 317 (1935).

(2) W. Mason and A. Buswell, "Examination of Water," page 50 (1938).

(3) U. S. Dispensatory, 22nd Edition, page 686.

(4) T. Sollman, "A Manual of Pharmacology," 5th Edition, page 485.

(5) Marvin R. Thompson, Marvin J. Andrews and Casimer T. Ichniowski, JOUR. A. PH. A., 22, 487–495 (1933).

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Books Make History—Even in Pharmacy†

Pharmacy in Retrospect, II

By Dr. George Urdang and F. W. Nitardy

The written word is the cherished memory of mankind. From olden days comes the significant sentence: "Quod non est in actis non est in mundo" (Whatever is not in the record, is not in the world); but until the time of the printing press, the written word was a limited privilege largely restricted to governments and learned men. Printing gave wings to words, gave publicity and distribution to thoughts and caused history-making results.

The "Squibb Ancient Pharmacy," that comprehensive collection of pharmaceutical objects of old, proves this also in the field of pharmacy. In two large table cases and on a corner shelf of the adjoining study—reminding us of that of Faust before he left God and science and surrendered to the devil, the world and Gretchen—numerous old books, manuscripts, edicts and franchises are awaiting those able and ready to commune with and enjoy these silent and yet so eloquent representatives of the past.

Fig. 1.—Incunabula—Ortolff's Arzneybuch printed 1479.

The two oldest books of the collection, printed before 1500 and therefore members of that illustrious group called "incunabula," are the "Hortus Sanitatis" (Garden of Health) printed in 1499 and the "Arzneybuch" (book of remedies), printed in 1479. It is most striking that they are not written in Latin, the language of the scientific world of those times but rather in the language of the people.

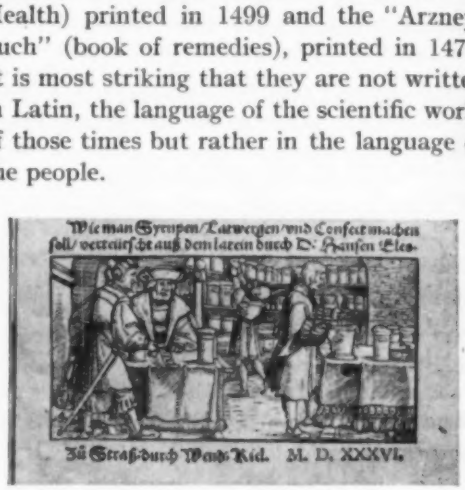

Fig. 2.—Woodcut showing 16th century pharmacy—from title page of "Reformation der Apothehen" printed 1536.

The people, that anonymous mass of men and women so unimportant in those days of absolutism, slowly came to the surface, through the aid of books, never to be submerged again. The knowledge presented by these early books was by no means new, but its dispersion beyond the jealously kept sphere of the learned few was new indeed, yes revolutionary.

In the preface to his Arzneybuch, the author says: "I, Ortolff Von Beyrlant, M.D., will write a book of remedies in the language of the people out of all remedy books I ever knew in Latin."

Striking also is the noble and classical print. (Fig. 1.) How well balanced are the paragraphs, the lines, and how fine the marginal proportions. In this product of the earliest printing press, we see a beauty of typography, scarcely reached and by no means surpassed by the best of our days; we feel therein the influence of the handsome handwriting as practiced by the monks in the holy seclusion of the medieval monasteries.

The "Hortus Sanitatis" and the "Arzneybuch" had many successors. Various her-

† Read by F. W. Nitardy before Section on History of Pharmacy, Atlanta meeting, August 23, 1939.

bals and formula books of the 16th, 17th and 18th centuries may be found in the collection; they are interesting but not revolutionary. The small book, "Reformation der Apotheken" (Reformation of the Pharmacies), may be considered a beginning of a new departure and in some respects a revolutionary one. Written by the famous physician and botanist, Otto Brunfels, and printed in 1536, it presents an account of pharmaceutical duties and responsibilities and gives detailed instructions. It was long a guide to both management and the authorities. The title page shows a most interesting woodcut, representing a pharmacy of that time. (Fig. 2.)

Shortly after the death of Brunfels, the practice of pharmacy became better standardized and more secure in its foundation through the introduction of official formularies. The new period was inaugurated by the Nuremberg Pharmacopœia of 1546 and the more representative Augsburg Pharmacopœia of 1564. Of that latter and most important work the Squibb collection contains the 1623 edition.

It may be recorded that Dr. Edward Kremers in 1926 edited and secured the publication of a facsimile print of the very rare first edition by the Hollister Pharmaceutical Library Fund of the State Historical Society of Wisconsin.

As Joseph Schafer, in his preface to the facsimile, justly remarks, there is no doubt that the Augsburg Pharmacopœia influenced the compilers of the London Pharmacopœia, later editions of which, in turn, served as a direct guide to the compilers of the first United States Pharmacopœia (1820). Thus this reproduction represents not only an important event, but also a noble gesture by which American science and generosity give thanks and recognition to the old world for its pioneering contribution in this field.

Just as the 1623 Augsburg Pharmacopœia is typical of the official materia medica of the 17th century, so is the best-known Wurtenberg Pharmacopœia (Fig. 3) in its editions of the second half of the 18th century quite representative for the materia medica of the whole 18th century.

In the last third of this turbulent century, the empirical, speculative and sometimes mysterious theories gradually gave way to knowledge resulting from the then-increasing scientific research. The "Pharmacopœia Wirtenbergica" of 1786, which we find in the Squibb collection, stands in the midst of this development. It not only contains

Fig. 3.—Würtenberg Pharmacopœia of 1786.

products which we still find almost unchanged in the present U. S. P. (mercury preparations, lead acetate, silver nitrate, tincture of iron chloride, etc.), but also many examples of the old and mysterious. A formula for prepared earthworms ("lumbricorum terrestrium praeparatio") reads, in shortened translation:

> "Living earthworms are to be washed until they are perfectly clean. Then the worms shall be macerated with wine for 4 hours and thereafter be quickly dried in an oven."

The product is recommended internally as an aperient, diuretic, diaphoretic and anodyne and to be used "bono cum successu" (with good success) against arthritis, convulsions and epilepsy.

Will their claimed, and at that time well-established, medical virtue cause modern researchers to examine earthworms and determine if, after all, they do harbor a constituent of real merit?

There is also a formula for foxoil ("oleum vulpinum"), directing to take an "intact and lively" fox, skin and eviscerate, then boil with six pounds of water, four pounds of old, clear oil and six ounces of salt. While boiling add a pound each of dill and thyme, boiling until the flesh separates from the bones and the water has evaporated. The

filtered oil then recovered is recommended for nervous attacks and stiff joints.

A formula for "sal volatile urinæ" a salt, to be prepared from the urine of young people, is mentioned but not recommended. The product, says the Pharmacopœia, is superfluous. Yet now we know of potent and valuable hormones available from urine of animals in certain stages of life.

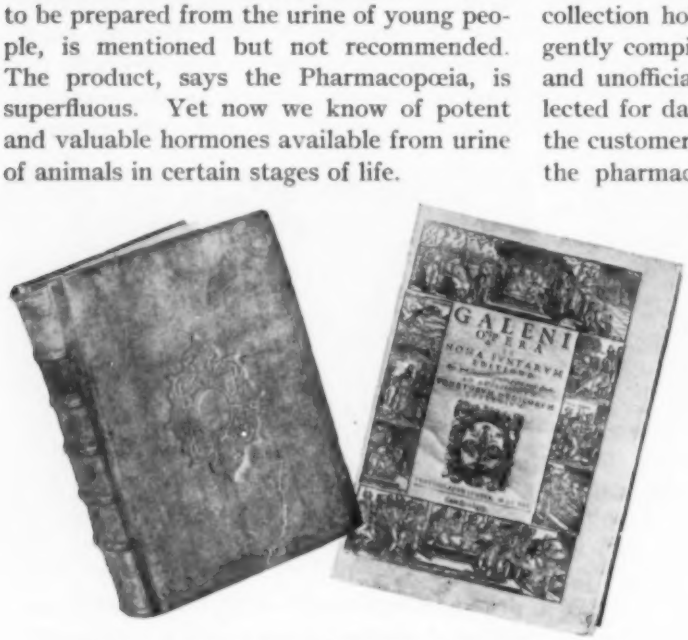

Fig. 4.—The works of Galen, one of the four volumes.

No doubt it was conservatism that caused a formula, thought to be superfluous, to be included. Conservatism, however, is not only a personal attribute but also a general one, and may sometimes even be the dangerous transfer of the physical law of inertia to the mental sphere.

Thus, the works of Galen, who lived 130–210 A.D., ruled the therapy of the civilized world for more than 1400 years. Galen devised a plausible system, and plausible systems often have great longevity. The Squibb collection includes the last of the nine editions (4 imposing volumes) which were published in Venice between 1541 and 1625. They represent the highest degree of medieval conservatism and splendor in medical literature. (Fig. 4.)

Conservatism, however often not scientific, is also the characteristic feature of the several handwritten formula books of the 17th and 18th centuries which the Squibb collection holds. These books contain diligently compiled formulas—personal, official and unofficial, serious and mysterious, collected for daily use, mute evidence of what the customers of that time wanted and how the pharmacist met their demands. The handwriting skill displayed in these goosequill written records is admirable indeed. (Fig. 5.)

From such hardwritten formula books, it is but a step to the old franchises. Some of them grant rights to produce and sell certain nostrums. The formulas for such nostrums, however, may often be traced back to the handwritten private formula book.

The Squibb collection has two of the finest examples of such nostrum privileges in existence. They are no mere documents but really books of many large pages of precious parchment, beautifully inscribed and splendidly bound.

Fig. 5.—Handwritten formula book.

Affixed to each, by means of heavy silken cords, are the great imperial seals, artistic creations of red wax in polished walnut cases.

Looking at the first pages of a franchise granted and signed in Vienna by the Emperor Carl VI, we may visualize the since departed glory of the Holy Roman Empire. Not less than seventy-three kingdoms, principalities and duchies figure in the titles following the name of the emperor; among them the kingdom of Hierusalem (Jerusalem), reminding us of the Crusades, and "The Terrae Firmæ"(Continents) "of the Oceanic Sea" (which includes the Americas). The possession of these lands had been lost long ago, but the titles still remained. (Fig. 6.)

Fig. 6.—Roval franchise for the production and sale of "life pills" signed by Carl VI of the "Holy Roman Empire." Title page, signature page and seal.

All that pompous display had only the purpose to license "Our and the Empire's faithful and dear David Liebel, citizen and merchant in Our's and the Holy Empire's town Nuremburg to prepare and to sell certain life pills," a secret medical composition, which, "according to credible testimonies and manifold approved experience are used with extremely good success against fever, plague, poison and other bad illnesses and needed in the Holy Empire, as well as in foreign kingdoms and countries."

Another franchise granted and signed in Vienna August 20, 1791, by Emperor Leopold II (Fig. 7) is a confirmation of an earlier franchise for the production and sale of "Trusinger's Healing Plaster," granted for the first time by Emperor Ferdinand III (1636–1657) and renewed by all his successors. Naturally each privilege or confirmation had to be highly paid for. Business must have been good for all parties concerned.

How different the relatively plain document by which Frederic the Great of Prussia

Fig. 7.—Royal franchise for the production and sale of "Trusinger's Plaster" signed by Emporer Leopold II, signature page of the large parchment document with seal.

(1740–1786) permitted in 1763 the establishment of a new pharmacy in Distorff by pharmacist Friedrich Wiesel. There is no superfluous phrase in the clear language of this franchise. We salute with respect the signature of this great king and man. (Fig. 8.)

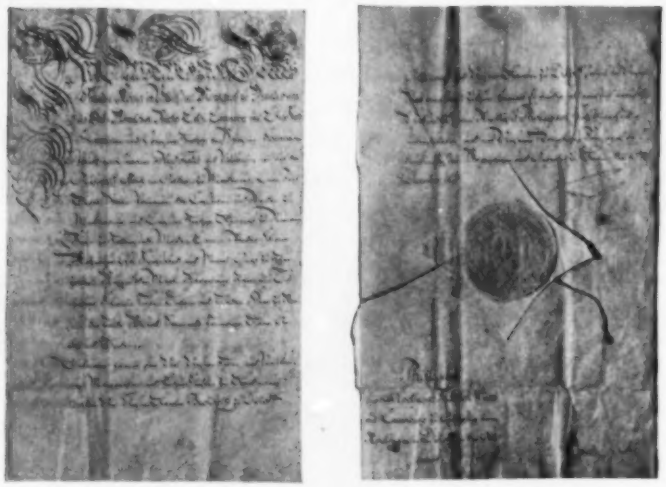

Fig. 8.—Franchise for the establishment of a new pharmacy signed by Frederick the Great of Prussia.

Emperors and Kings!—It would seem that here the Pope cannot be overlooked. Indeed this inexhaustible collection does provide a pharmaceutical document given and signed by Pope Leo XII in 1824—a bound parchment permitting the establishment of a pharmacy by Dominus Paulus Ruga in Rome. (Fig. 9.)

Fig. 9.—Franchise for the establishment of a Pharmacy signed by Pope Leo XII.

Aside from these books and official documents, the Squibb collection contains many others, among them a number of general edicts concerning the practice of pharmacy of previous centuries, and some schedules of government-established prices for remedies. Such authoritatively fixed prices are legal to the present day in many European countries, thereby eliminating the, to us all too familiar, "price cutting" with all its evils. Freedom also has its price.

Books, old franchises, manuscripts! All pertaining to pharmacy, and yet a part of the universal history with which we are all connected, by which we are all affected and in which we all have a part. It is told Archimedes once said: "Give me a firm place on which to stand and from it I shall move the earth." *For us, this firm place is Pharmacy!*

Pharmacy as a Subject of Religious Influence and Feeling*

Pharmacy in Retrospect, III

By Dr. George Urdang and F. W. Nitardy†

In early times illness was believed to be a divine punishment or—as in the case of Hiob—a divine temptation, and hence it was considered up to God to withdraw it again. So it is not surprising to find in those times the rôle of apothecary, physician and priest combined in practice as a single profession, and among the primitives even to-day, of course, the "medicine man" is still the potent combination of priest, apothecary and physician.

Long after the healing or care of body and soul had become separate professions, tradition of the old combination still shed its glory over pharmacy. The Christian Church considered the supply of remedies to the poor as a part of its religious duties, and scientific pharmacy of the Middle Ages was widely practiced by the clergy. In the monasteries the practice of pharmacy was an important occupation, and it was here especially where medicinal and aromatic herbs were diligently cultivated, extracted and distilled. The monks copied the old

Fig. 1.—Della Robbia bas-relief, Santa Barnaba Church.

* A brief pharmaceutico-historical discussion of the subject from earliest times to about the 18th to 19th centuries illustrated with lantern slides of pharmaceutico-religious objects, mostly from the Squibb Ancient Pharmacy.

† Read by F. W. Nitardy before Section on History of Pharmacy, Atlanta meeting, August 23, 1939.

medical books, the works of Galen, of Plinius, Serapion, Mesue and especially of Dioscorides, annotated and amplified them on the basis of their own experience. They developed formulas and combinations which acquired great renown and some of which have been preserved and are used to this day.

The mundane pharmacists of the Middle Ages took possession of the scientific pharmaceutical work of the Church as well as of the religious background of their profession. They were convinced that Providence had purposefully provided mankind with rich and varied natural sources of useful and healing agents. It was the task of pharmacy and its practitioners not only to develop this great gift of nature, to isolate, purify, perfect and preserve the curative and beneficial substances, but also to maintain and, if necessary, defend the ethics and ideals of their noble profession, ideals about which there is something truly divine.

With such a background, and the fervent piety of the plain people naturally reflected in the learned professions, of which pharmacy was one, it is not surprising to find the close harmony of Christian and pharmaceutical ideals and ethics given expression through the fine arts and in the symbolism, decoration and naming of pharmacies and apothecaries' shops of previous centuries.

Fig. 2.—Large wood carving, Mary with Jesus.

Each country and period of time had its deities to whom the origin of the healing arts was attributed and who at the same time were its patrons (Isis, Osiris and later

on Thoth in old Egypt; Bacchus, Apollo Ammon and Aesculapius in old Greece, and so on).

With Christianity, the saints and martyrs succeeded the pagan deities. Often we find the Virgin Mary looked upon as the protectress of pharmacy. In the 15th century, the Virgin Mary was the patroness of the combined guild of physicians and pharmacists in Florence. There are two sculptures, originating from the workshop of the famous artists, the brothers della Robbia, which have transmitted this fact to posterity. One, a medallion on the south gable of the church Orsamichele without any inscription, shows the Virgin with the Child surrounded by plants; the other, a bas-relief in the church Santa Barnaba, bears the inscription "Sub gubernatione artis aromatariorum." Thus this work of art must have been dedicated by the pharmaceutical profession. (Fig. 1.)

In Central Europe, where the custom of naming the pharmacies after persons, animals, planets, etc., has continued to the present, many a pharmacy, especially in Catholic Bavaria, bears the name "Marienapotheke" (Virgin Mary Pharmacy) and has its front or its interior adorned with sculptures or pictures of the Virgin. Cloister pharmacies, especially, often have chosen this patroness and decorated their walls with statues or paintings portraying the Mother of Christ. The "Squibb Ancient Pharmacy" contains a large wood carving of Mary holding in her arms the dead Jesus, a carving which once was the holy sign and symbol of an old cloister pharmacy. (Fig. 2.)

Fig. 3.—Cosmas and Damien—page from Dutch Legend Book.

Other saints, too, such as Jacobus the Senior, Nicholas of Pari, Lucas, Michael, Raphael, Rochus and finally Mary Magdalen, were more or less often adopted as patrons of individual pharmacies and pharmaceutical guilds. But these may be considered as mainly of local importance. Recognized in the whole Christian world as the patrons of pharmacy, were the saints Cosmas and Damian; famous through the service, as pharmacist and physician, they gave so freely, without payment, to every one. The two brothers died in 303 as martyrs, during the persecution of the Christians by Emperor Diocletian.

Fig. 4.—Oil painting—Christ as Apothecary.

These early victims of intolerance were so highly regarded in the Christian world of that time that churches bearing their names were erected to honor them; statues, sculptures and pictures glorify them, and numerous pamphlets and legend-books from the 5th to the 19th centuries describe their life and martyrdom. Most of the works of art devoted to Cosmas and Damian show one brother as pharmacist and the other as physician, the former holding a drug or ointment container and a spatula and the latter a flask or urine glass, that old symbol of medicine. A page out of a 16th-century Dutch legend-book devoted to the martyrs, which we find in the "Squibb Ancient Pharmacy" (Fig. 3), shows a woodcut represent-

ing the brothers, and pictures them as described.

From the Middle Ages to the 18th century, the religious aspects of the pharmacy were by no means restricted to individual pharmacies or pharmacists. The Christian creed and the fear of God were in those times indispensable requirements for the practice of pharmacy in most countries of Christendom. We still find in the Royal Prussian and Electoral Brandenburgian Medical Edict of 1725, a copy of which is also to be found in the "Squibb Ancient Pharmacy," a definite stipulation that pharmacists must profess belief in the Christian Faith. There is scarcely one book in the long row of old herbals or other pharmaceutical works contained in the Squibb collection without a preface in which the author invokes God.

Thus, it is not astonishing that even the equipment of the pharmacies often bore witness to the piety of their owners. The Squibb collection possesses a number of jars, adorned with paintings of religious nature, and several mortars bearing the pious admonition to praise or to love God above all.

The most significant and symbolic of these expressions of the unity of Christian ideals and pharmaceutical ethics and practices may be found in the paintings representing Christ as Apothecary of the Soul. Of about seventy examples of paintings of this motif known to-day, the "Squibb Ancient Pharmacy" is the proud possessor of one of the most beautiful. It is a large 18th-century oil painting, German in origin, rich in color and most harmoniously composed. (Fig. 4.) The figure of the Saviour dominates the picture. His right hand is raised heaven-ward and in his left hand he holds the old-fashioned hand-dispensing balance. Light rays emanate from his head, giving a figure which is most impressive. Naturally all objects to be seen in that pious picture have their symbolic value. The word "Gerechtig-keit" (righteousness) underneath the hand-balance shows that the Saviour dispenses justice. The chalice bears the word "Glaube" (faith). The herb on the right side symbolizes with its designation "Tag und Nacht" the omnipresence of God; the herb in the foreground with its appellation "Kreutzwurtzl" (cross-root) suggests the crucifixion. The drug containers on the table carry inscriptions representing the more important Christian virtues, among them charity, constancy and hope. Quotations of Christ's word appearing to both sides of the figure and at the bottom of the picture invite all to seek here the assistance needed. The deep meaning of this picture may be read on the book lying on the table in the foreground, "Himmelsbuch der Arzney" (Heaven's Book of Remedy). A drawing of identical composition and design may

Fig. 5.—Gold and Silver Votive Shrine in form of a Pharmacy.

be found in the Swiss National Museum; it is not known at this time if it represents a copy of the painting, the artist's drawing or possibly a sketch by an earlier artist which inspired a later one to reproduce it in oil.

Dr. Fritz Ferchl, a well-known German authority in the field of pharmaceutical objects of art, has made a special study of such pictures. According to him, such representations were largely painted between the 16th and 19th centuries in the Germanic countries; they fall into Catholic and Protestant series; typical of the latter are quotations from Christ's sayings taken from Luther's translation of the Bible, as is the case in this painting. On the other hand, the Host shown above the chalice is frequently a Catholic symbol.

Fig. 6.—Pair of cherubs representing a portion of the decorative elements of the baroque fixtures of the Pharmacy.

Further evidence of public appreciation of the high ideals of professional pharmacy and its then pious character may be found in the "Squibb Ancient Pharmacy." The Catholic custom of making votive gifts, continued through centuries as a visible ex-

pression in return for divine grace and help in special cases of pain and disease, has led to the creation of a magnificent religio-pharmaceutical symbol which we may now admire in the Squibb collection: a tabernacle pharmacy embossed in silver and gold. (Fig. 5.)

This "pharmacy," once bequeathed as a thanksgiving to the real pharmacy of a Bavarian monastery, is a late baroque work of art, in all probability from the hands of an Augsburg gold- or silversmith of the middle of the 18th century. Especially in Augsburg around that time, this artistic craftsmanship had its second flowering. The drug drawers, pots and boxes are a sublime piece of work. In the upper arch we see openings used to hold candles, whose gentle light illumined this unique flower of religious pharmacy. Here Christ is not represented as pharmacist, but we find the crucifix in the foreground.

One of the most famous pictures painted by Titian shows the incarnation of celestial and mundane love, separating and at the same time combining them. In a similar way, the Squibb collection adds to its witnesses of celestial love, also one which deals with the terrestrial variety. Two shields held by cherubs, a part of the beautiful and antique baroque fixtures of the "Squibb Ancient Pharmacy" (Fig. 6) (*a* and *b*) proclaim the sentence: "Amor nullis herbis medicabilis est" which means: Love cannot be cured by herbs. The herbs provided through divine love of man for the treatment of disease thus were not intended to assuage the pangs of terrestrial love. If the herbs cannot ease the pain of love, what can? Time, fate, God?

> "Name it then as you will . . .
> Feeling is all in all;
> Name is but sound and fog
> O'er clouding heaven's glow."
>
> (Goethe, Faust, Part I.)

Book Reviews

Salben und Salben Grundlagen (Ointments and Ointment Bases). A guide for physicians and pharmacists. By DR. H. V. CZETISCH-LINDENWALD and DR. F. SCHMIDT-LABAUME; the former, an apothecary at the Biolaboratory of I. G.-Dye Industry; the latter, lecturer and head of Skin Disease Clinic of the Hospital of Mannheim, with a contribution on the ointments for the protection of the skin of the workers in the industries by R. Jäger of the Institute for Colloidal Research of the Johan Wolfgang Goethe University, Frankfurt a M. Published by Julius Springer, Berlin. Octavo, 240 pages, 36 illustrations. Price, R. M. 16.80 unbound; bound, R.M. 18.

The general arrangement begins with an historical résumé, followed by a classification of the ointments according to the constituents, animal fats, waxes, mineral oils; alcohols, emulsions, prescriptions; definitions for ointments and pastes. The special part treats of uses, bases, etc. with discussions as to what is desired in ointment preparations—are they to be absorbed, cooling or protective, how practical application is to be made; the aromatic and antiseptic values; the tests relative to color, keeping qualities, etc. The authors have given careful study to the various phases and then made experimental application. This part of the work can be commended, but it is somewhat limited with regard to the ointments used in this country. Vitamins in ointments have been studied with regard to medicinal value, determination of medicinal ingredients, tar, sulfur, mercurials. The authors have cautioned the users of the book relative to dangerous products, for example, ointments containing radioactive substances. This discussion is very helpful and is evidently an original presentation. Throughout the authors have sought to give practical information. The volume will be a valuable addition to the libraries of medical, pharmacy and veterinary schools. The last chapter is devoted to methods of application.—E. G. EBERLE.

Fundamentals of Pharmacy, by WALTER H. BLOME, Wayne University College of Pharmacy, Detroit, Mich., and CHARLES H. STOCKING, University of Michigan, College of Pharmacy, Ann Arbor, Mich. with a chapter on Hospital Pharmacy by EDWARD C. WATTS, University of Michigan Hospital, Ann Arbor, Mich. Octavo, 364 pages; 157 illustrations. Price, cloth, $4.50. Publishers, Lea and Febiger Philadelphia.

The volume is dedicated to Alviso Burdette Stevens, councilor, teacher, co-worker and friend. The authors state "That the book has been written to fill the growing need for a text that should treat fundamentals of Pharmacy in concise form and yet be complete enough to supply the essential information of the art of preparing, standardizing and preserving drugs and medicines." They have made use of their experiences and drawn upon the historical knowledge during years of Association mem-

bership. A textbook on Pharmacy must, to be of value, have an informative background; "Fundamentals on Pharmacy" is based on the latest revised edition of the U. S. Pharmacopœia, U. S. P. XI. A treatise, aside from conciseness should present the subject in a way which distinguishes it from other works on the subject and thereby establishes a reason for its production and acceptance. The authors and co-workers are well and favorably known and this gives the book an introduction; it is hoped that it will prove its value and will establish a worthwhile reason for its creation and continuance over long periods of successive revisions.—E. G. EBERLE.

Neues Manual für die Praktische Pharmazie (New Manual for Practical Pharmacy, Second Edition), by DR. MAX SIDO, druggist. Publisher, Julius Springer, Berlin, Germany, 1938. Bound, 274 pages. Price, R.M. 15.

The Table of Contents shows the extensive study of the author, who is a practical druggist and pharmacist of high standing; for some time associated with the "Pharmazeutische Zeitung," discontinued with number 104 of the year 1937, and he has brought into the Manual the formulas of that publication and preserved them in that way. The Manual contains a wide variety of formulas and indicates that the druggist has demands which may enlarge the volume of sales; evidently these possibilities are not very different from those in this country, but depend on the individuals. The titles listed in the Table of Contents indicate the variety of formulas; among them are, formulas of Face Preparations, perfumed cosmetics and other complexion preparations for hands, lips, nails, mouth, shaving, massages, teeth, household remedies, confections, antiseptics, veterinary remedies, moth destroyers, inks, dyes, cleaning preparations. The author has been associated with a druggist's publication and is well and favorably known in German pharmacy.—E. G. EBERLE.

Die Tablettenfabrikation und ihre maschinellen Hilfsmittel (Tablet Manufacture and the Machine), by DR. GEORGE ARENDS and DR. J. ARENDS. Fourth revised Edition, 1938. 53 illustrations. 220 pages. Publisher, Julius Springer, Berlin, W. 9, Linkstrasse. Price, R.M. 12,

While the references given are of value it cannot be stated that they are complete. Tablet making in Germany seems to have had its beginning in 1872, when Professor Rosenthal, of Erlangen, described his tablet compressor. Tablet manufacture began somewhat earlier in the United States (See JOUR. A. PH. A., 1914, page 820) and many articles on the subject have been published in this country which are not referred to in the book. The authors have prepared a valuable publication and drawn upon their experience in its preparation.—E. G. EBERLE.

What's Your Allergy? by DR. LAURENCE FARMER and GEORGE HEAXER. 230 pages. Publisher, Random House, 20 E. 57th St., N. Y. Published, October 24, 1939. Price, $2.00.

Allergy is a word given to the American speaking people within this twentieth century but its definition embodies maladies which are centuries old. Such familiar afflictions as hay fever, asthma, eczema, hives and catarrh are a few of the maladies classified under the definition of allergy. "What's Your Allergy?" tells what allergies are and how they may be combatted. The authors have written for the patient and the layman for clear and concise reading in a manner novel to a book of this kind It is all told in six chapters. Idiosyncrasy was long accepted as an explanation for provocations of hay fever and others. But with Professor Richets' work a new theory was given for these maladies known as anaphylaxis, also a word only slightly older than the word allergy itself. Allergy emerges with the provocations conceived after years of extensive research. Diagnosis and treatment of the provocations are expounded only as a possible means of cure. This book is recommended as an interesting presentation of such allergies as hay fever, eczema, hives, catarrh, etc., to patient and layman.—EMERSON C. BEELER.

Merck's Index, Fifth Edition. Published by Merck & Co., Inc., Rahway, N. J. Price, $3.00.

The First American Edition of Mercks Index was published in 1889; to some extent it had the character of a price-list and it was explained that the prices were given as a guide. The nomenclature and orthography were those followed by the Chemical Society of England; it is stated that this issue is a guide for the physician, apothecary, chemist and dealer. The Second Edition is designated an encyclopœdia for the physician and pharmacist; more attention is given to definitions, physiological effects and therapeutic uses and description; the information given is for the pharmaceutical dispenser. Omitting reference to intervening editions, the Fifth is an encyclopœdia of a thousand pages and contains useful information on chemicals and drugs, antidotes and poisons. These data are well arranged and carefully studied by professional workers, qualified for the work, prepared by D. W. Coutlee, Joseph Rosin, C. R. Addinall, Alfred I. Cone. The Index contains also records of 4500 Chemical, Clinico-Chemical Reactions, tests; formulas of culture media, fixatives, staining solutions, etc. In the Section on Coal-Tar Colors for use in Foods, Drugs and Cosmetics, 113 colors are described. The information given makes this volume of value to pharmacists, physicians, dentists, veterinarians, chemists and manufacturers. The character of the Index has changed with each issue, and its appeal has likewise changed. The book is well-bound, the paper and typography good and will be found a useful reference book for the libraries of those mentioned in the preceding lines and of others, and in fact, there is a rightful place for it in most libraries.—E. G. EBERLE.